A METHOD FOR IMPROVED CHEMICAL SYNTHESIS OF GUANIDINIUM ALKALOIDS

Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

FIELD OF THE INVENTION

The present invention relates to methods for improved synthesis of guanidinium alkaloids, and more particularly to the total, convergent synthesis of the Crambescidin/Ptilomycalin family of guanidinium alkaloids.

This invention was made with Government Support under Grant No. NIH NHLBIS (HL-25854), awarded by the National Institutes of Health. The Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

Crambe crambe, a bright red encrusting sponge commonly found at shallow depths along the rocky coast of the Mediterranean is a rich source of structurally novel, bioactive alkaloids (Figure 1). Among the most remarkable marine guanidine natural products are the family of alkaloids depicted in Figure 1 that have a rigid pentacyclic guanidine carboxylic acid core linked to an ω-hydroxycarboxylic acid, ester or polyamine amide. This family, exemplified by ptilomycalin A (compound **1**), the crambescidins (compounds **2-6**), celerômycalin and fromiamycalin (compound **10**) are characterized by a structurally unique pentacyclic guanidinium core that has a spermidine or hydroxyspermidine residue tethered by a long chain ω–hydroxycarboxylic acid spacer.

The alkaloid, ptilomycalin A, was reported by Kashman, Kakisawa and co-workers from

sponges collected in the Caribbean and Red Sea (Kashman et al., J. Am. Chem. Soc., 1989, 111:8925). Ptilomycalin A exhibits cytotoxicity against P388 (IC_{50} 0.1 μg/mL), L1210 (IC_{50} 0.4 μg/mL) and KB (IC_{50} 1.3 μg/mL), antifungal activity against *Candida albicans* (MIC 0.8 μg/mL) as well as considerable antiviral activity against *Herpes simplex* virus, type 1 (HSV-1) at a concentration of 0.2 μg/mL (Overman, L. E.; et al. *supra*). Recently, ptilomycalin A has been shown to inhibit the brain Na^+, K^+ -ATPase and Ca^{2+} -ATPase from skeletal sarcoplasmic reticulum with IC_{50} values of 2μM and 10μM, respectively (Ohtani, I.; et al.. *Euro. J. Pharm.* **1996**, *310*, 95).

In addition to Ptilomycalin A, numerous other complex marine alkaloids having a hydropyrrolo[1,2-c]pyrimidine-4-carboxylate part structure have been isolated including 13,14,15-isocrambescidin 800, crambescidin 800 and crambescidin 816 from *Crambe crambe* (Jares-Erijman et al., J. Org. Chem. 1991, 56:5712-5715; Jares-Erigman et al., J. Org. Chem. 1993, 58:4805-4808; Tavares et al., Biochem. Syst. Ecol., 1994, 22:645-646; Berlinck et al., J. Nat. Prod. 1993, 56, 1007-10015.)

Ptilomycalin A and several of the crambescidins show substantial antitumor, antiviral and antifungal activities. Crambescidin alkaloids have been described for use in inhibition of calcium channels (Jares-Erijman, et al., J. Org. Chem. 1993, 58:4805); inhibition of Na^+, K^+ and Ca^{2+}-ATPases (Ohizumi et al., Eur. J. Pharmacol., 1996, 310:95). Batzelladine alkaloids, exemplified by batzelladines B and D (Figure 1, Patil et al., J. Org. Chem., 1995, 60:1182; Patil et al., J. Org. Chem. , 1997, 62:1814; and Patil et al., J. Nat. Prod., 1997, 60:704), are reported to modulate protein-protein interactions that are important for immunological responses (Patil et al., 1995 and J. Org. Chem., 1997, supra).

As a result of its low abundance, 13,14,15-isocrambescidin 800 has not been extensively screened, although it is reported to be less cytotoxic to L-1210 cells than other crambescidins. (Jares-Erijman et al., J. Org. Chem., 1993, 58:4805-4808, supra).

The defining structural feature of the crambescidin alkaloids is a pentacyclic guanidine unit linked by a straight chain ω-hydroxycarboxylic acid tether to a spermidine or hydroxyspermidine unit. Extensive NMR studies demonstrated that the relative stereochemistry of the pentacyclic cores of crambescidin 800, crambescidin 816 and ptilomycalin A is identical (Jares-Erijman et al., supra and Tavares et al., supra), while 13,14,15-isocrambescidin 800 is epimeric at C13, C14 and C15 relative to other members of the crambescidin family (Jares-Erijman et al., J. Org. Chem., 1993, supra, and Berlinck et al., J. Nat. Prod., supra). The absolute configuration of the guanidine moieties of 13,14,15-isocramescidin 800 and crambescidin 816 was established by oxidative degradation of the oxepene rings of these alkaloids to yield (*S*)-2-hydroxybutanoic acid (Jares-Erijman et al., J. Org. Chem., 1993, supra), while the absolute configuration of the hydroxyspermidine unit of crambescidin 816 was assigned using Mosher's method (Berlinck et al., supra, and Dale et al., J. Am. Chem. Soc., 1973, 95:512-519). Since ^{1}H NMR and ^{13}C NMR chemical shifts in the hydroxyspermidine fragments of 13,14,15-isocrambescidin 800 are nearly identical to those of 2 and 3, it had been assumed that the stereochemistry at C43 is the same for all Crambescidins (Berlinck et al., supra).

Apparent in the alkaloid compounds described (Figure 1), is the occurrence of the hydropyrrolo [1,2c]pyrimidine unit with either the syn or anti relationship of the hydrogens flanking the pyrrolidine nitrogen.

In 1893, Biginelli reported the synthesis of dihydropyrimidines from the condensation of ethyl acetoacetate, aromatic aldehydes and urea. (Biginelli, P., Gazz. Chem. Ital., 1893, 23:360 (1893). Since Biginelli's disclosure, variations in all three components have led to the synthesis of an array of functionalized dihydropyrimidines and analogues. (Kappe, C. O., Tetrahedron, 49:6937 (1993). In 1993, we reported on the viability of "tethered Biginelli" condensations and verified that the *cis* orientation of the methine hydrogens was preferentially realized when the dehydrative condensation was promoted under Knoevenagel conditions to form cisi-1-oxohexahydropyrollo[1,2-c]pyrimidine products. (Overman et al., J. Org. Chem., 1993, 58:3235-3237). These reactions represented the first use of the Biginelli

reactions in stereocontrolled organic synthesis. Tethered Biginelli condensations have already proved to be powerful reactions for the construction of Crambescidin (Overman et al., J. Am. Chem. Soc., 1995, 117:265) and batzelladine alkaloids (Franklin et al., J. Org. Chem., 1999, 62:6379). Recently it was reported to use acetals in place of alkenes to generate the aldehyde component of a Biginelli cyclization (Cohen et al., Organic Letters, 1999, V1 N13:2169-2172).

In 1995, an enantioselective total synthesis of (–)-Ptilomycalin A (Overman et al., J. Am. Chem. Soc., 117:2657 (1995)) was reported, which was the first total synthesis of a member of the Crambescidin alkaloid family.

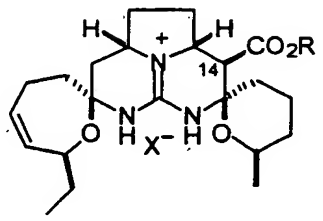
There remains a need for improved methods of total, convergent synthesis of alkaloid compounds having biological activity, such as antifungal, antiviral and/or anti-tumor activity.

SUMMARY OF THE INVENTION

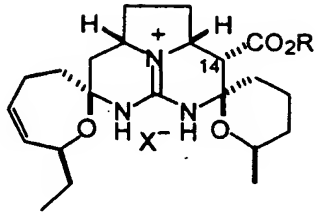
Accordingly, the present invention provides improved methods for convergent, total enantioselective synthesis of guanidinium alkaloid compounds including compounds having *cis*- or *-trans*-1-oxo-and 1-iminohexahydropyrrolo [1,2-c]pyrimidine units such as, 13,14,15-Isocrambescidin 800, Crambescidin 800 and Ptilomycalin A, for use as therapeutic agents having antifungal, antiviral and/or antitumor activity.

The compounds of the invention may be represented by the formulae:

COMPOUNDS I-V.



I



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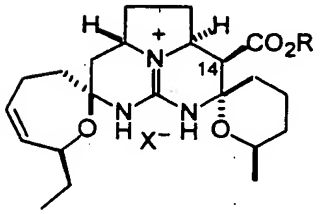

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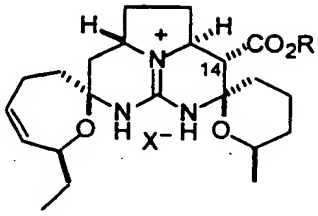

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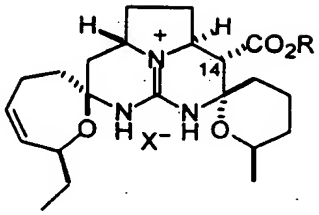
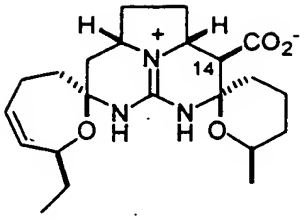


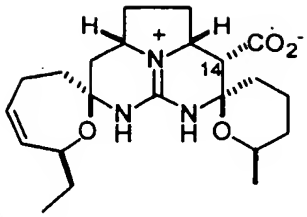
V

In which R= H, a carboxylic acid protecting group, an ω-alkoxycarboxylic acid or an ω-alkoxycarboxylic acid ester, and X= any pharmaceutically acceptable counterion.

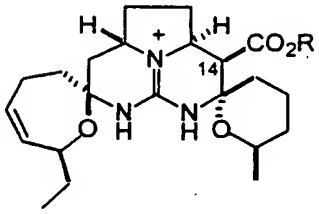
COMPOUNDS IA-VA



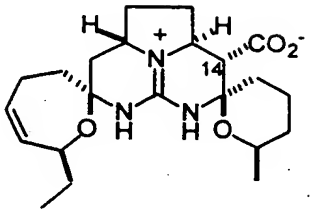
I-A



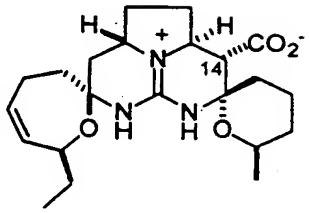
II-A



III-A

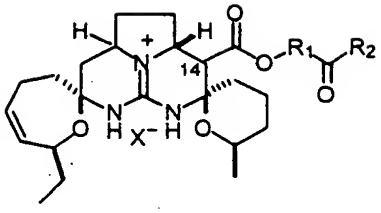


IV-A

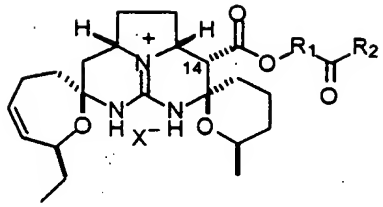


V-A

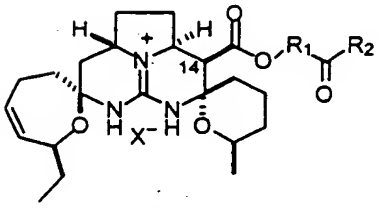
COMPOUNDS VI-X



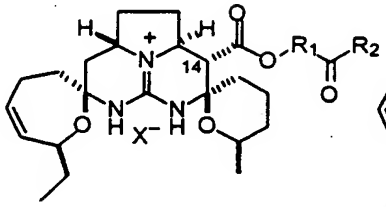
VI



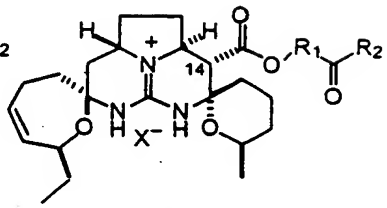
VII



VIII



IX



X

In which, R_1= any alkyl, aryl or substituted alkyl group, R_2= O⁻, OH, OG_1, a spermidine moiety or a substituted spermidine moiety, where G_1 = a carboxylic acid protecting group and X= any pharmaceutically acceptable counterion.

The methods of the invention employ a convergent strategy for obtaining the compounds of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts pentacyclic marine guanidine alkaloids obtained from marine organisms.

Figure 2 depicts a molecular mechanics model of the Ptilomycalin A/Crambescidin core.

Figure 3 depicts a hexahydropyrrolopyrimidine (compound **B**) having trans stereochemistry prepared by the methods of the invention.

Figure 4 illustrates Biginelli condensations of tethered ureido aldehydes using the methods of the invention, as described in Example I, infra.

Figure 5 is a synthetic scheme for making compounds **23-24**, as described in Example I, infra.

Figure 6 is a synthetic scheme for making compounds **25-28**, as described in Example I, infra..

Figure 7 presents two hypotheses for tethered Bignelli condensations under Knoevenagel conditions (Y=OH or NR_2).

Figure 8 illustrates syntheses for compounds **37 - 43** as described in Example II, infra.

Figure 9 depicts reactions for synthesis of Ptilomycalin A (compounds **46** and **47**) as described in Example II, infra.

Figure 10 depicts syntheses of compounds **49** to **53** as described in Example II, infra.

Figure 11 depicts syntheses of compounds **54** to **56**, as described in Example II, infra.

Figure 12 illustrates syntheses of compounds **58** and **54** from compounds **57** and **59**, as described in Example II, infra..

Figure 13 illustrates the syntheses of compounds **61 – 68** and Ptilomycalin A , as described in Example II, infra.

Figure 14 is a model showing expected preference for axial addition in forming the oxepene ring, as described in Example II, infra.

Figure 15 depicts the syntheses of Crambescidin 800 (compound **2**) and compounds **71 – 75**, as described in Example III, infra.

Figure 16 depicts the syntheses of compounds **76** to **80**, as described in Example III, infra.

Figure 17 depicts the syntheses of compounds **81** to **84**, as described in Example III, infra.

Figure 18 depicts the syntheses of compounds **85** to **88**, as described in Example III, infra.

Figure 19 depicts the syntheses of compounds **89** to **93**, and compound **2** (Crambescidin 800), as described in Example III, infra.

Figure 20 is a molecular mechanics model of the 13, 14, 15-Isocrambescidin 800 core and the Ptilomycalin A/Crambescidin core, as described in Example IV, infra.

Figure 21 is a retrosynthetic analysis of the Isocrambescidin core, as described in Example IV, infra.

Figure 22 depicts syntheses of compounds **99-103** as described in Example IV, infra.

Figure 23 depicts syntheses of compounds **105a-106** as described in Example IV, infra.

Figure 24 shows the pentacyclic intermediate in the (-)-Ptilomycalin A synthesis as described in Example IV I, infra.

Figure 25 shows the synthesis of compounds **105a and 105b** through **108a and 108b**, as described in Example IV, infra.

Figure 26 shows the synthesis of Isocrambescidin 800 (compound **2**) as described in Example IV, infra.

Figure 27 depicts the creation of compounds **114-116** as described in Example IV, infra.

Figure 28 depicts the synthesis of compound **117**, as described in Example IV, infra.

Figure 29 shows data for Mosher's derivatives of compounds **10** and **117** as described in Example IV, infra.

Figure 30 is a scheme showing a Biginelli condensation between a tethered guanyl aldehyde and a β-ketoester afforded 1-iminohexahydropyrrolo[1,2-*c*]pyrimidine intermediates with the *trans* relationship about the pyrrolidine ring thus providing a strategy for constructing the Isocrambescidin core.

Figure 31 is three-dimensional models of methyl ester analogs of four pentacyclic guanidine units as described in Example V, infra. Models depicting only heavy atoms are oriented identical to the line drawings; models also showing hydrogen atoms are oriented with the guanidine unit projecting back.

Figure 32 is a scheme showing a Biginelli condensation between a guanyl aldehyde (or aminal) and a β-ketoester afforded 1-iminohexahydropyrrolo[1,2-*c*]pyrimidine intermediates with the *trans* relationship about the pyrrolidine ring thus providing a strategy for constructing the Isocrambescidin core.

Figure 33 shows the retrosynthesis of the pentacyclic core of Isocrambescidin 800 (compound **10**) as described in Example V, infra.

Figure 34 depicts the synthesis of compound **134**, as described in Example V, infra.

Figure 35 depicts the formation of Pentacycle **135**, as described in Example V, infra.

Figure 36 depicts the formation of Pentacycle **135** using pyridinium *p*-toluenesulfonate, as described in Example V, infra.

Figure 37 depicts the formation of Pentacycle **135** using HCl, as described in Example V, infra.

Figure 38 depicts models of the methyl ester analog of **139** showing the two chair conformations of the hydropyran ring. In conformation A, the methyl group is axial and in conformation B it is equatorial.

Figure 39 depicts the formation of Penacycle **135b** using DCl, as described in Example V, infra.

Figure 40 depicts the formation of compounds **141-143**, as described in Example V, infra.

Figure 41 depicts the formation of compounds **141-143** from compound **138**, as described in Example V, infra.

Figure 42 depicts the formation of compounds **145-146**, as described in Example V, infra.

Figure 43 depicts the formation of compounds **141-147**, as described in Example V, infra.

Figure 44 depicts the F-19 NMR data for Mosher derivatives of compounds **10** and **147**, as described in Example V, infra.

Figure 45 depicts the relative energy of pentacyclic guanidine isomers, as described in Example V, infra.

Figure 46 is a schematic diagram for a modified enantioselective total synthetic approach.

Figure 47 is a schematic diagram of the tethered Biginelli condensation.

Figure 48 is an improved synthetic approach of compound **152**.

Figure 49 is a schematic diagram for making enantiopure Iodide compound **166**, as described in Example VI, infra.

Figure 50 is a schematic diagram for coupling of the C(1)–C(7) fragment with the tricyclic intermediate, as described in Example VI, infra.

Figure 51 is a schematic diagram for making a pentacyclic acid as described in Example VI, infra.

Figure 52 is a schematic diagram for an improved method for making pentacyclic acids, as described in Example VII, infra.

Figure 53 is a diagram showing the synthesis of compounds **180** to **183** as described in Example VII, infra.

Figure 54 is a depiction of the synthesis of compounds **185-189** as described in Example VII, infra.

Figure 55 is a depiction of the synthesis of compound **194** as described in Example VII, infra.

Figure 56 is a mean graph response of Ptilomycalin A as described in Example VIII, infra.

Figure 57 is a mean graph response of Isocrambescidin 800 trihydrochloride as described in Example VIII, infra.

Figure 58 is a mean graph response of Triacetylcrambescidin 800 chloride as described in

Example VIII, infra.

Figure 59 is a mean graph response of Crambescidin 657 hydrochloride as described in Example VIII, infra.

Figure 60 is a mean graph response of Crambescidin 800 trihydrochloride as described in Example VIII, infra.

Figure 61 is a mean graph response of Triacetylisocrambescidin 800 chloride as described in Example VIII, infra.

Figure 62 is a mean graph response of 13-Epiptilomycalin A as described in Example VIII, infra.

DETAILED DESCRIPTION OF THE INVENTION

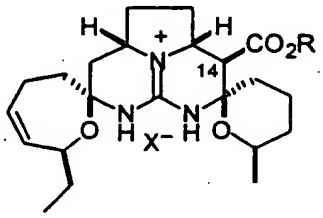
The present invention provides methods for enantioselective total synthesis of guanidinium alkaloids and congeners in convergent fashion using tethered Biginelli reactions. This invention allows all of the heavy atoms of the pentacyclic core of the Crambescidin/Ptilomycalin A and Isocrambescidin to be assembled in one key step. The compounds produced may be used for pharmacological screening using known methods, to identify compounds having desired biological therapeutic activity, for example as antiviral, antifungal and/or antitumor agents.

For synthesizing the compounds of the invention, a method was developed for controlling stereoselection in tethered Biginelli condensations to synthesize either the *cis* or *trans* stereoisomer of 1-oxo and 1-iminohexahydropyrrolo[1,2-c]pyrimidines.

The invention also provides methods for the preparation of pentacyclic acid (e.g., compound **7** of Figure 1) and precursor allyl ester (e.g., compound **8** of Figure 1) intermediates, that allows

analogs to be prepared that are not available by degradation of the sponge extracts (Kashman, Y.; et al. *J. Am. Chem. Soc.* **1989**, *111*, 8925; Ohtani, I.; et al. *J. Am. Chem. Soc.* **1992**, *114*, 8472; Jares-Erijman, E. A.; et al. *J. Org. Chem.* **1991**, 56, 5712). It is expected that analogs will show improved pharmacological properties.

The present invention relates to compounds of the general formulae:



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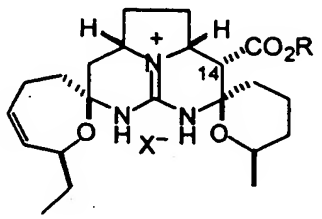

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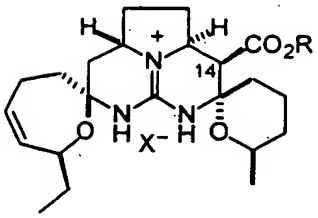

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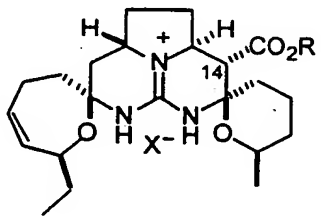

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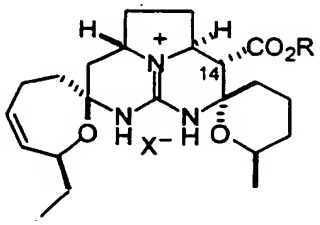


V

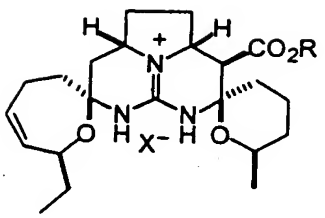
In which R= H, a carboxylic acid protecting group, an ω-alkoxycarboxylic acid or an ω-alkoxycarboxylic acid ester, and X= any pharmaceutically acceptable counterion.

In one embodiment R= H and X=Cl^-.

In another embodiment R= allyl and X=Cl^-.

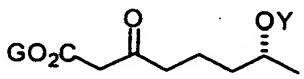
In another embodiment R= $(CH_2)_{15}CO_2H$ and X = Cl^-.

The invention includes methods for preparing the compounds. In a method for preparing compound I having the formula:

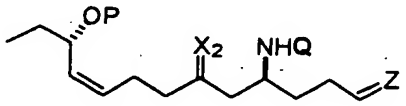


in which R= H, a carboxylic acid protecting group, an ω-alkoxycarboxylic acid or an ω-alkoxycarboxylic acid ester, and X= any pharmaceutically acceptable counterion

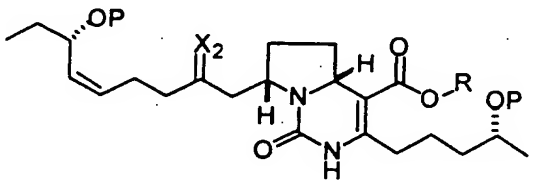
a compound having the formula:



in which G= a carboxylic acid protecting group, an ω-alkoxycarboxylic acid or and ω-alkoxycarboxylic acid ester, and Y= alcohol protecting group, is reacted with a compound of the formula:

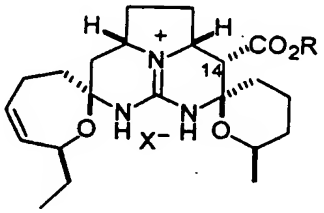


In which X_2= O or a ketone protecting group, Z= alkene or carbonyl protecting group, P= alcohol protecting group, and Q= amino carbonyl group, to produce a compound of the formula:



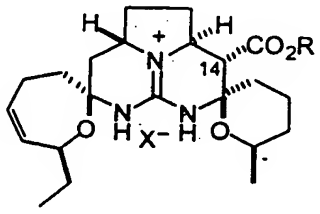
in which X_2= O or ketone protecting group, P= alcohol protecting group, and R= carboxylic acid protecting group, ω-alkoxycarboxylic acid or ω-alkoxycarboxylic acid ester which is subsequently converted to the pentacyclic compound by deprotection, incorporation of ammonia, and cyclization.

Another embodiment is a method for preparing compound **II**:

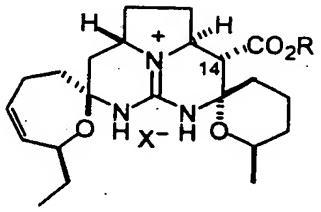


In which, R= H, a carboxylic acid protecting group, an ω-alkoxycarboxylic acid or an ω-alkoxycarboxylic acid ester, and X= any pharmaceutically acceptable counterion, by epimerizing the stereocenter at carbon-14 of the compound **I**.

In another embodiment, a method for preparing compounds **IV** and **V**:

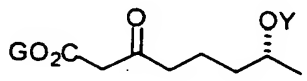


IV

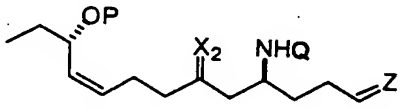


V

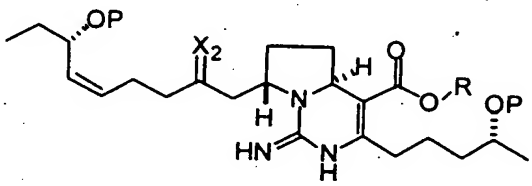
in which R= H, a carboxylic acid protecting group, an ω-alkoxycarboxylic acid or an ω-alkoxycarboxylic acid ester, and X= any pharmaceutically acceptable counterion by reacting compound



in which G= carboxylic acid protecting group, an ω-alkoxycarboxylic acid or an ω alkoxycarboxylic acid ester, and Y= alcohol protecting group, with compound

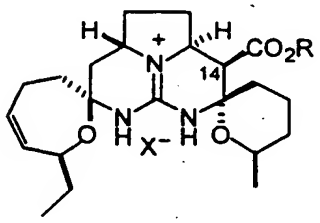


In which X_2= O or ketone protecting group, Z= alkene or carbonyl protecting group P= alcohol protecting group, and Q= amidinyl group to produce a compound of the formula



In which X_2= O or ketone protecting group, P= alcohol protecting group, R= carboxylic acid protecting group, an ω-alkoxycarboxylic acid or an ω-alkoxycarboxylic acid ester which is subsequently converted **IV** and **V** by deprotection and cyclization.

Another embodiment is a method for preparing compound **III**:



In which R= H, a carboxylic acid protecting group, an ω-alkoxycarboxylic acid or an ω-alkoxycarboxylic acid ester, and X= any pharmaceutically acceptable counterion by epimerizing the stereocenter at carbon-14 and carbon 15 of the compound **IV**.

Protecting groups and strategies for synthesis of organic compounds are well known in the art (Protective Groups in Organic Synthesis, 2nd Ed. T.W. Greene, P.G.M. Wuts, J. Wiley and Sons, Inc. New York, 1991).

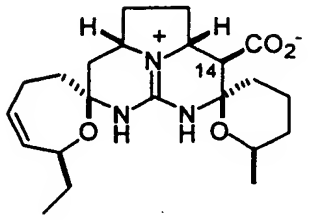
Carboxylic acid protecting groups may be chosen from the following groups including, but not limited to, esters and amides.

Alcohol protecting groups may be chosen from the following groups including, but not limited to, ether groups, silyl protecting groups, such as TIPS, TBDMS, SEM, THP, TES, TMS, or ester groups, such as acetates, benzoates, and mesitoates.

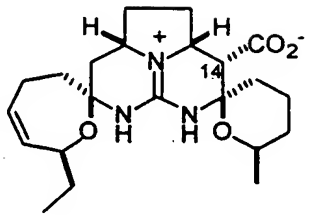
Carbonyl protecting groups may be chosen from the following groups including, but not limited to, ethers, cyclic or acyclic acetals, ketals, thioketals or thioacetals.

Amine protecting groups may be chosen from the following groups including, but not limited to, N-alkyl, such as benzyl, methyl, N-Silyl groups, N-acyl groups, N-carbamates.

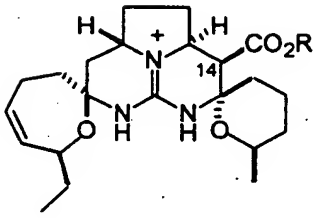
The invention also provides compounds of the general formulas:



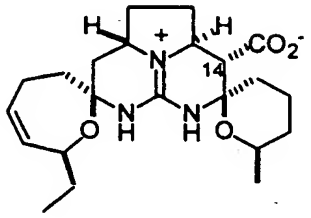
I-A



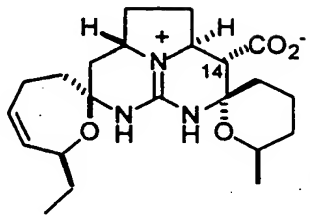
II-A



III-A



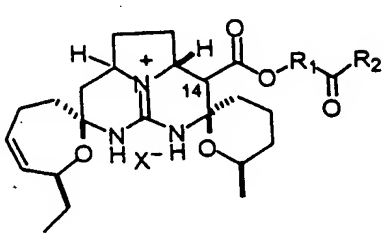
IV-A



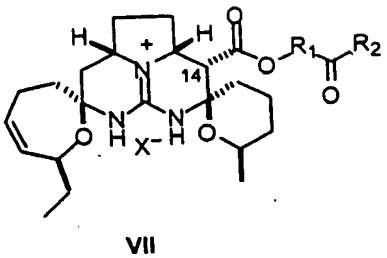
V-A

Another embodiment is a method for preparing compounds **I-A** to **V-A**, which can be prepared by following the method of preparing compounds **I** to **V**, respectively, and including an additional step of removing the carboxylic acid protecting group (R) or deprotecting the carboxylic acid.

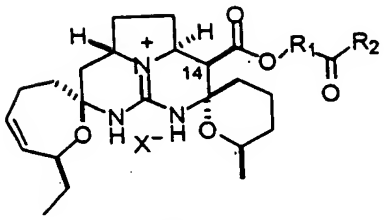
Further, the invention provides compounds having the formula:



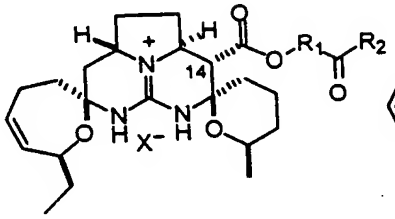
VI



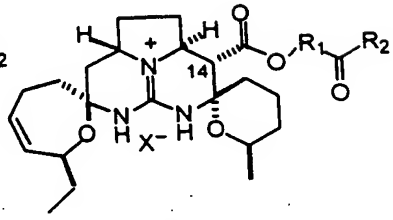
VII



VIII



IX

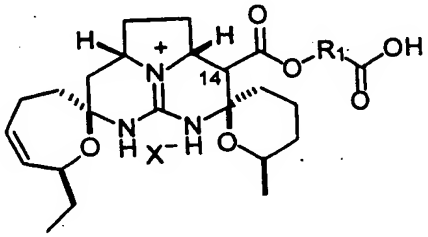


X

In which, R_1= any alkyl, aryl or substituted alkyl group, R_2= O^-, OH, OG_1, spermidine moiety or substituted spermidine moiety, in which G_1 =carboxylic acid protecting group and X= any pharmaceutically acceptable counterion

Methods for preparing compounds **VI-X:**

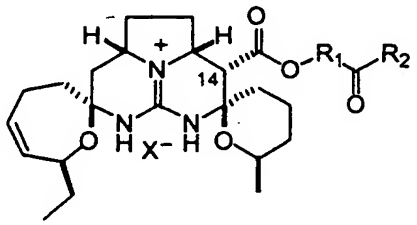
Compound **VI** is prepared as described for compound **I** in which R is an ω-alkoxycarboxylic acid as depicted in the figure below:



In which R_1= any alkyl, aryl or substituted alkyl group and including an

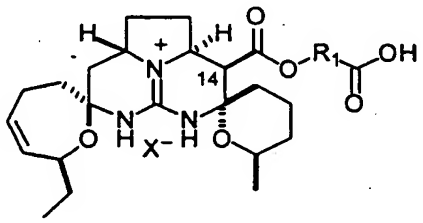
additional step of reacting the pentacyclic compound of the formula above with a protected spermidine or a protected substituted sperimidine and subsequently deprotecting to produce **VI**.

Compound **VII** is prepared as described for compound **II** in which R is an ω-alkoxycarboxylic acid acid as depicted in the figure below:



In which R_1= any alkyl, aryl or substituted alkyl group and including an additional step of reacting the pentacyclic compound of the formula above with a protected spermidine or a protected substituted sperimidine and subsequently deprotecting to produce **VII**.

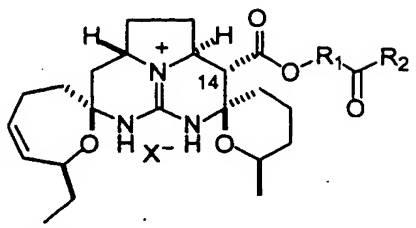
Compound **VIII** is prepared as described for compound **III** in which R is an ω-alkoxycarboxylic acid acid as depicted in the figure below:



In which R_1= any alkyl, aryl or substituted alkyl group and including an additional step of reacting the pentacyclic compound of the formula above with a protected spermidine or a protected substituted sperimidine and subsequently deprotecting to produce **VIII**.

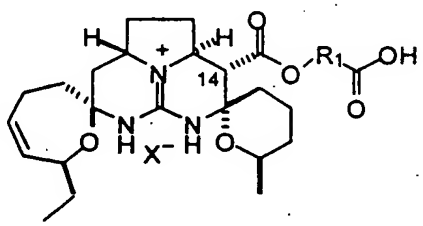
Compound **IX** is prepared as described for compound **IV** in which R is an ω-

alkoxycarboxylic acid acid as depicted in the figure below:



In which R_1= any alkyl, aryl or substituted alkyl group and including an additional step of reacting the pentacyclic compound of the formula above with a protected spermidine or a protected substituted sperimidine and subsequently deprotecting to produce **IX**.

Compound **X** is prepared as described for compound **V** in which R is an ω-alkoxycarboxylic acid acid as depicted in the figure below:



In which R_1= any alkyl, aryl or substituted alkyl group and including an additional step of reacting the pentacyclic compound of the formula above with a protected spermidine or a protected substituted sperimidine and subsequently deprotecting to produce **X**.

The compounds of the invention include where applicable, a geometric or optical isomer of the compound or racemic mixture thereof.

Pharmaceutically acceptable counterions may be chosen from the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate,

methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate.

The compounds of the invention may be used in therapy as antiviral, antifungal and/or as itumor agents. For such uses, the compounds are administered intravenously, intramuscularly, topically, transdermally by means of skin patches, bucally, suppositorally or orally to man or other animals. The compositions can be presented for administration to humans and animals in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions, granules, sterile parenteral solutions or suspensions, oral solutions or suspensions, oil in water and water in oil emulsions containing suitable quantities of the compound, suppositories and in fluid suspensions or solutions. The preferred form depends upon the mode of administration and the therapeutic application.

For oral administration, either solid or fluid unit dosage forms can be prepared. For preparing solid compositions such as tablets, the compound can be mixed with conventional ingredients e.g. talc, magnesium stearate, dicalcium phosphate, magnesium aluminum silicate, calcium sulfate, starch, lactose, acacia, methylcellulose, and functionally similar materials as pharmaceutical diluents or carriers. Capsules are prepared by mixing the compound with an inert pharmaceutical diluent and filling the mixture into a hard gelatin capsule of suitable size. Soft gelatin capsules are prepared by machine encapsulation of a slurry of the compound with vegetable oil, light liquid petrolatum or other inert oil.

Dosage forms for oral administration include syrups, elixirs, and suspensions. The forms can be dissolved in an aqueous vehicle along with sugar, aromatic flavoring agents and preservatives to form a syrup. Suspensions can be prepared with an aqueous vehicle with the aid of a suspending agent for example acacia, tragacanth, methylcellulose and the like.

For parenteral administration, fluid unit dosage forms can be prepared utilizing the compound and a sterile vehicle. In preparing solutions the compound can be dissolved in the vehicle for injection and filter sterilized before filling into a suitable vial or ampoule and sealing. Adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. The composition can be frozen after filling into a vial and the water removed under vacuum. The dry lyophilized powder can then be sealed in the vial and reconstituted prior to use.

The most effective mode of administration and dosage regimen for the molecules of the present invention depends upon the severity and course of the disease, the subject's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the molecules should be titrated to the individual subject.

Adjustments in the dosage regimens and/or modes of administration may be made to optimize the antiviral, antifungal or antitumor efficacy of the compounds of the invention.

Efficacy of the compounds of the invention in therapy may be assessed using known methods. For example, efficacy of the compounds as anti-tumor agents may be assessed by tumor biopsy or non-invasive procedures to determine tumor growth inhibition. Similarly, efficacy of the compounds as anti-viral or anti-fungal agents may be determined using standard protocols such as as assays to detect decreases in numbers of viral particles or fungal cells, or in the numbers of virally or fungally infected cells.

The following examples are presented to illustrate the present invention and to assist one of

ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

EXAMPLE I

Synthesis of *cis*- or *trans*-1-Oxo-and 1-Iminohexahydropoyrrolo[1,2-c]pyrimidines

This Example describes a method for controlling the stereoselectivity of tethered Biginelli condensations. Modification of the electrophilic reaction component permits access to hexahydropyrrolopyrimidines (Compound **10** in Figure 3) having either the *cis* or *trans* stereochemistry.

Materials and Methods

The strategy for synthesis of compounds **12-18** is depicted in Figure 4, for compounds **21-24** in Figure 5 and for compounds **25-28** in Figure 6. Methods of synthesis were used as previously disclosed and known in the art, e.g. Minor and Overman, J. Org. Chem., 1997, 62:6379, incorporated by reference herein.

Synthesis of (R)-Benzyloxy-7-methyloct-6-en-3-ol (Compound 12). A solution of (*R*)-methyl-3-hydroxy-7-methyl-6-octenoate (Kitamuram et al., Org. Synth., 1992 71:1) (21.5 g, 0.115 mol) and Et_2O (100 mL) was added dropwise to a 0°C suspension of $LiAlH_4$ (6.8 g, 0.18 mol) and Et_2O (0.5 L). After 1 h, H_2O (6.8 mL), 3 M NaOH (6.8 mL), and H_2O (20.4 mL) were added sequentially. The resulting mixture was filtered through a pad of Celite, the filtrate was concentrated, and the resulting oil was purified on silica gel (1:1 hexanes-EtOAc) to provide 13.8 g (76%) of (*R*)-7-methyloct-6-ene-1,3-diol as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.04-5.08 (m, 1H) 3.82 (s, 2H) 3.68-3.79 (m, 3H) 1.97-2.05 (m, 2H) 1.59-1.67 (m, 4H) 1.54-1.60 (m, 4H) 1.40-1.48 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) 131.8, 123.8, 70.8, 60.7, 38.3, 37.5, 25.5, 24.1, 17.5 ppm; IR (film) 3356 cm^{-1}; $[\alpha]^{23}_D$ +3.5, $[\alpha]^{23}_{577}$ +4.5, $[\alpha]^{23}_{546}$ +4.7, $[\alpha]^{23}_{425}$ +7.3, $[\alpha]^{23}_{405}$ +8.1, (*c* 1.2, $CHCl_3$). Anal. Calcd for $C_9H_{18}O_2$: C,

68.31; H, 11.47. Found: C, 68.09; H, 11.54.

A solution of (*R*)-7-methyloct-6-ene-1,3-diol (7.00 g, 44.3 mmol) and DMF (80 mL) was added dropwise to a -40°C suspension of NaH (3.20g, 133 mmol, prewashed with hexanes 3 x 50 mL) and DMF (130 mL). After 15 min, benzyl bromide (5.30 mL, 44.3 mmol) was added, and the reaction was warmed to -10°C over 1 h. The reaction was quenched by pouring into saturated aqueous NH_4Cl (300 mL), and the resulting mixture was extracted with Et_2O (4 x 150 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), and filtered, and the filtrate was concentrated. The crude oil was purified on silica gel (9:1 hexanes-EtOAc to 4:1 hexanes-EtOAc) to provide 7.74 g (71%) of **12** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.36 (m, 4H) 7.26-7.31 (m, 1H) 5.14-5.17 (m, 1H) 4.51 (s, 2H) 3.78-3.83 (m, 1H) 3.66-3.73 (m, 1H) 3.62-3.65 (m, 1H) 3.04 (s, 1H) 2.05-2.16 (m, 2H) 1.73-1.77 (m, 2H) 1.71 (s, 3H) 1.63 (s, 3H) 1.44-1.57 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) 137.8, 131.5,128.2, 127.5, 127.4, 124.0, 73.0, 70.4, 68.8, 37.3, 36.3, 25.5, 24.0, 17.5 ppm; IR (film) 3443 cm^{-1}; $[\alpha]^{23}_D$ + 13.0, $[\alpha]^{23}_{577}$ +13.9, $[\alpha]^{23}_{546}$ +15.6, $[\alpha]^{23}_{435}$ +26.5, $[\alpha]^{23}_{405}$ +31.3 (*c* 1.4, $CHCl_3$). Anal. Calcd for $C_{16}H_{24}O_2$: C, 77.38; H, 9.74. Found: C, 77.25; H, 9.74.

Synthesis of (S)-3-Amino-1-benzyloxy-7-methyl-6-octene (compound 13). Diethyl azodicarboxylate (4.12 g, 23.7 mmol) was added dropwise to a solution of **13** (5.05 g, 20.3 mmol), Ph_3P (6.22 g, 23.7 mmol), HN_3 (12 mL, 2.0 M in toluene), and toluene (75 mL) at 0°C. After 15 min, hexanes (0.2 L) was added, the resulting mixture was filtered through a plug of silica gel (the plug was washed with 30 mL of hexanes), and the eluent was concentrated to yield the crude azide as a slightly yellow oil that was used without further purification.

A solution of this crude azide and Et_2O (20 mL) was added dropwise to a stirred 0°C suspension of $LiAlH_4$ (0.91 g, 24.0 mmol) and Et_2O (100 mL), and after 15 min the reaction was warmed to room temperature. After 1 h, the reaction was cooled to 0°C, and H_2O (1 mL), 3 M NaOH (1 mL), and H_2O (3 mL) were added sequentially. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated to provide 4.53 g (90%) of

amine **13** as a colorless oil that was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.38 (m, 4H) 7.27-7.32 (m, 1H) 5.11-5.14 (m, 1H) 4.52 (s, 2H) 3.56-3.65 (m, 2H) 2.88-2.95 (m, 1H) 2.00-2.12 (m, 2H) 1.74-1.82 (m, 1H) 1.70 (s, 3H) 1.62 (s, 3H) 1.42-1.60 (m, 2H) 1.21-1.37 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 138.4, 131.5, 128.3, 127.5, 127.4, 124.1, 72.9, 68.1, 48.8, 38.4, 37.6, 25.6, 24.6, 17.6 ppm; IR (film) 3366 cm^{-1}; $[\alpha]^{23}_D$ -3.3, $[\alpha]^{23}_{577}$ -2.7, $[\alpha I^{23}_{546}$ -3.2, $[\alpha]^{23}_{435}$ -4.9, $[\alpha]^{23}_{405}$ -6.3 (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{16}H_{25}NO$-HCl: C, 67.71; H, 9.23; N, 4.93. Found: C, 67.68; H, 9.27; N, 5.00.

Synthesis of (S)-l-Benzyloxy-7-methyl-3-ureido-6-octene (Compound 14b). Trimethylsilyl isocyanate (0.90 mL, 6.7 mmol) was added to a solution of crude **13** (1.15 g, 4.65 mmol) and *i*-PrOH (7 mL) at room temperature. After 4 h, the reaction was concentrated, and the resulting oil was purified on silica gel (3:1 hexanes-EtOAc to EtOAc) to provide 873 mg (65%) of **14b** as a colorless solid: mp 79-81°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.36 (m, 5H) 5.45 (s, 1H) 5.08-5.11 (m, 1H) 5.93 (s, 2H) 4.94 (s, 2H) 3.53-3.63 (m, 3H) 2.05 (m, 2H) 1.83-1.90 (m, 1H) 1.69 (s, 3H) 1.60 (m, 4H) 1.42-1.54 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) 159.4, 138.0, 131.8, 128.3, 127.6, 127.5, 123.6, 72.9, 67.3, 47.9, 35.7, 35.3, 25.6, 24.4, 17.6 ppm; IR (film) 3340, 1653, 1602 cm^{-1}; $[\alpha]^{23}_D$ +16.0, $[\alpha]^{23}_{577}$ +17.3, $[\alpha]^{23}_{546}$ +19.6, $[\alpha]^{23}_{435}$ +34.5, $[\alpha]^{23}_{405}$ +42.6 (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{17}H_{26}N_2O_2$: C, 70.31; H, 9.02; N, 9.65. Found: C, 70.39; H, 9.09; N, 9.55.

Conversion of Compound 14a to Intermediate Ia with Ozone. Ozone was bubbled through a solution of urea **14a** (120 mg, 0.60 mmol), CH_2Cl_2 (5 mL), and MeOH (1 mL) at -78°C until the solution was saturated (blue color appeared and persisted for 10 min). Nitrogen was then bubbled through the solution to remove excess ozone, Ph_3P-polystyrene (550 mg, 3 mmol P/g resin) was added, and the reaction was allowed to warm to room temperature. After 2 h, the reaction mixture was filtered, morpholinium acetate (140 mg, 0.90 mmol) was added to the filtrate, and the resulting solution was concentrated to give a colorless oil that was used without further purification.

Representative Procedure for Biginelli Condensation under Knoevenagel Conditions. Conversion of Compound 1a to 17 and 18a. A solution of crude aminal **1a** (0.60 mmol), benzyl acetoacetate (0.16 mL, 0.90 mmol), morpholinium acetate (140 mg, 0.90 mmol), and 2,2,2-trifluoroethanol (0.6 mL) was maintained at 60°C for 2 d. After being cooled to room temperature, the reaction was partitioned between Et_2O (20 mL) and 50% aqueous NH_4Cl (5 mL). The layers were separated, the organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated. The resulting oil was purified on silica gel (2:1 hexanes-EtOAc to 1:1 hexanes-EtOAc) to give 126 mg (64%) of **17a** and 32 mg (16%) of **18a**.

(4aR,7S)-7-(2-Hydroxyethyl)-3-methyl-1-oxo-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxylic acid benzyl ester (17a): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H) 7.29-7.35 (m, 5H) 5.10-5.20 (m, 2H) 4.25 (dd, J = 11.3, 4.7 Hz, 1H) 4.11 (dd, J = 13.8, 8.2 Hz, 1H) 3.84 (s, 1H) 3.56 (m, 2H) 2.43-2.48 (m, 1H) 2.22 (s, 3H) 2.02-2.08 (m, 1H) 1.81-1.87 (m, 1H) 1.65-1.74 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 165.6, 154.9, 149.3, 135.9, 128.5, 128.3, 128.1, 102.2, 65.9, 59.0, 58.4, 52.2, 39.3, 30.6, 29.8, 18.0 ppm; IR (film) 3356, 1707, 1673, 1627 cm^{-1}; $[\alpha]^{23}_D$ -26.5, $[\alpha]^{23}_{577}$ -26.8, $[\alpha]^{23}_{546}$ -37.1, $[\alpha]^{23}_{435}$ -119, $[\alpha]^{23}_{405}$ -184 (*c* 1.00, $CHCl_3$); HRMS (CI) *m/z* 331.1657 (MH, 331.1658 calcd for $C_{18}H_{23}N_2O_4$).

(4aS,7S)-7-(2-Hydroxyethyl)-3-methyl-1-oxo-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxylic acid benzyl ester (18a): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (s, 1H) 7.30-7.38 (m, 5H) 5.12-5.22 (m, 2H) 4.42 (m, 1H) 4.35 (dd, J = 10.2, 4.5 Hz, 1H) 4.33-4.44 (br s, 1H) 3.60 (m, 2H) 2.40-2.45 (m, 1H) 2.45 (s, 3H) 2.06-2.10 (m, 1H) 1.76-1.84 (m, 1H) 1.39-1.55 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 165.8, 153.0, 146.0, 136.1, 128.6, 128.6, 128.1, 99.1, 65.9, 58.9, 57.3, 53.6, 38.3, 34.9, 28.2, 18.3 ppm; IR (film) 3377, 3232, 1713, 1682, 1633 cm^{-1}; $[\alpha]^{23}_D$ -29.2, $[\alpha]^{23}_{577}$ -29.0, $[\alpha]^{23}_{546}$ -31.0, $[\alpha]^{23}_{435}$ -30.2 (*c* 1.05, $CHCl_3$); HRMS (CI) *m/z* 331.1629 (MH, 331.1658 calcd for for $C_{18}H_{23}N_2O_4$). Anal. Calcd for $C_{18}H_{22}N_2O_4$: C, 65.44; H, 6.71; N, 8.48.

Represenative Procedure for Generating Tethered Biginelli Precursors by Dihydroxylation and 1,2-Diol Cleavage. Conversion of 14b to 15. Osmium tetroxide (0.4 mL, 0.1 M in *t*-BuOH) was added to a solution of **14b** (120 mg, 0.41 mmol), *N*-methylmorpholine *N*-oxide (230 mg, 1.96 mmol), pyridine (30 mL, 0.4 mmol), and 10:1 THF-H_2O (8 mL). After 30 min, Florisil (1 g), $NaHSO_3$ (1 g), and EtOAc (20 mL) were added, and the resulting mixture was stirred. After 30 min, the reaction mixture was filtered, and the filtrate was concentrated to provide the corresponding 1,2-diol as a colorless oil that was used without further purification.

A solution of this crude diol, $Pb(OAc)_4$ (0.21 g, 0.48 mmol), and CH_2Cl_2 (8 mL) was maintained for 30 min at room temperature. The reaction mixture was then filtered through a plug of Celite, morpholinium acetate (92 mg, 0.62 mmol) was added to the filtrate, and this solution was concentrated to provide crude aminal **15** as a slightly yellow oil (Garigipati et al., J. Am. Chem. Soc., 1985, 107:7790).

Conversion of Compound 15 to 17b and 18b under Knoevenagel Biginelli Conditions. Following the representative procedure for Biginelli condensation under Knoevenagel conditions, crude aminal **15** (0.41 mmol) was condensed with **16**, and the crude product was purified on silica gel (2:1 hexanes-EtOAc to 1:1 hexanes-EtOAc) to provide 140 mg (81%) of a 4:1 mixture of **17b** and **18b**. The isomers were separated by medium-pressure liquid chromatography (MPLC) on silica gel (2:1 hexanes-EtOAc to 1:1 hexanes-EtOAc).

(4aR,7S)-7-(2-Benzyloxyethyl)-3-methyl-1-oxo-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxylic acid benzyl ester (17b): ^1H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H) 7.25-7.38 (m, 10H) 5.11-5.21 (m, 2H) 4.43-4.53 (m, 2H) 4.28-4.31 (m, 1H) 3.98-4.02 (m, 1H) 3.51-3.55 (m, 2H) 2.43-2.48 (m, 1H) 2.22-2.28 (m, 1H) 2.20 (s, 3H) 1.86-1.95 (m, 2H) 1.74-1.78 (m, 1H) 1.61-1.66 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) 165.9, 152.7, 148.9, 138.4, 136.1, 128.5, 128.3, 128.3, 128.1, 127.5, 127.4, 101.4, 72.6, 67.8, 65.8, 58.0, 54.4, 33.4, 30.6, 28.9, 18.2 ppm; IR (film) 1682, 1633 cm^{-1}; $[\alpha]^{23}_D$ -18.7, $[\alpha]^{23}_{577}$ -20.3, $[\alpha]^{23}_{546}$ -25.0, $[\alpha]^{23}_{435}$ -71.7, $[\alpha]^{23}_{405}$ -108 (*c* 1.4, $CHCl_3$). Anal. Calcd for $C_{25}H_{28}N_2O_4$: C, 71.41; H,

6.71; N, 6.66. Found: C, 71.31; H, 6.80; N, 6.69.

(4aS,7S)-7-(2-Benzyloxyethyl)-3-methyl-1-oxo-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxylic acid benzyl ester (18b): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94 (s, 1H) 7.33-7.40 (m, 9H) 7.26-7.32 (m, 1H) 5.14-5.24 (m, 2H) 4.47-4.56 (m, 2H) 4.33-4.41 (m, 2H) 3.60-3.62 (m, 2H) 2.42-2.47 (m, 1H) 2.26 (s, 3H) 2.00-2.12 (m, 2H) 1.73-1.79 (m, 1H) 1.44-1.55 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) 166.0, 151.8, 147.1, 138.4, 136.3, 128.4, 128.2, 128.0, 127.9, 127.5, 127.4, 98.2, 72.8, 67.7, 65.5, 57.2, 54.6, 35.2, 34.8, 28.1, 18.2 ppm; IR (film) 1681, 1640 cm^{-1}; $[\alpha]^{23}_D$ -37.5, $[\alpha]^{23}_{577}$ -37.0, $[\alpha]^{23}_{546}$ -39.7, $[\alpha]^{23}_{435}$ -34.5, $[\alpha]^{23}_{405}$ -14.1 (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{25}H_{28}N_2O_4$: C, 71.41; H, 6.71; N, 6.66. Found: C, 71.30; H, 6.73; N, 6.59.

Representative Procedure for Biginelli Condensation in the Presence of PPE. Conversion of Compound 14b to 17b and 18b. Urea **14b** (115 mg, 0.400 mmol) was converted to **15** following the general olefin dihydroxylation and 1,2-diol cleavage procedure. A solution of the resulting crude aminal **15**, benzyl acetoacetate (110 mg, 0.59 mmol), polyphosphate ester (0.2 mL), and CH_2Cl_2 (0.2 mL) was maintained at room temperature for 2 d. The reaction was then quenched by adding Et_2O (20 mL) and 50% aqueous $NaHCO_3$ (5 mL). The layers were separated, the organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated. The resulting oil was purified on silica gel (2:1 hexanes-EtOAc to 1:1 hexanes-EtOAc) to provide a 101 mg (60%) of a 4:1 mixture of **18b** and **17b**.

(4aS,7S)-7-(2-Hydroxyethyl)-1-imino-3-methyl-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxylic acid benzyl ester hydroformate (Compound 23). Following the general procedure of Bernatowicz, (Bernatowicz et al., J. Org. Chem. 1992, 57:2497), a solution of (*S*)-3-amino-7-methyl-6-octenol (Overman et al., J. Am. Chem. 1995, 117:2657) (0.95 g, 6.0 mmol), 1*H*-pyrazole-1-carboxamidine hydrochloride (0.95 g, 6.1 mmol), *i*-Pr_2EtN (1.1 mL, 6.3 mmol), and DMF (2.7 mL) was heated at 60°C. After 4 h, the reaction mixture was concentrated, and the resulting crude **21**, a colorless oil, was used without further purification.

Ozone was bubbled through a solution of this sample of crude 21 and MeOH (25 mL) at -78°C until the solution was saturated. Nitrogen was then bubbled through the solution to remove excess ozone, Me_2S (1 mL) was added, and the reaction was allowed to warm to room temperature. After 1 h, the reaction mixture was dried ($MgSO_4$) and filtered, and the filtrate was concentrated to give **22** as a yellow oil that was used without further purification.

Following the representative procedure for Biginelli condensation under Knoevenagel conditions, aminal **22** was condensed with compound **16** and the crude product was purified on silica gel (100% $CHCl_3$ to 10:1 $CHCl_3$-*i*-PrOH to 10:1:0.1 $CHCl_3$-*i*-PrOH-HCO_2H) to yield 0.95 g (42%) of *trans*-Biginelli product **23** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.03 (br s, 2H) 8.29 (s, 2H) 7.27-7.35 (m, 5H) 5.19 (d, J = 12.3 Hz, 1H) 5.12 (d, J= 12.3 Hz, 1H) 4.28-4.38 (m, 2H) 3.76-3.78 (m, 1H) 3.49-3.53 (m, 1H) 2.45-2.50 (m, 1H) 2.28 (s, 3H) 2.11-2.17 (m, 1H) 1.81-1.87 (m, 1H) 1.58-1.67 (m, 2H) 1.47-1.54 (m, 1H), the OH signal was too broad to observe; ^{13}C NMR (125 MHz, $CDCl_3$) 166.6, 164.9, 150.7, 143.8, 135.5, 128.5, 128.2, 128.1, 101.1, 66.2, 57.1, 56.1, 56.0, 36.0, 34.1, 28.0, 17.2 ppm; IR (film) 3180, 1684, 1572 cm^{-1}; $[\alpha]^{23}_D$ -30.7, $[\alpha]^{23}_{577}$ -32.2, $[\alpha]^{23}_{546}$ -35.7 (*c* 3.1, $CDCl_3$); HRMS (FAB) *m/z* 330.1820 (MH, 330.1818 calcd for $C_{18}H_{24}O_3N_3$).

(4aS,7S)-1-(4-Bromobenzoylimino)-7-[2-(4-bromobenzoyloxy)ethyl]-3-methyl-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxylic Acid Benzyl Ester (24). 4-Bromobenzoyl chloride (400 mg, 1.81 mmol) was added at 0°C to a solution of **23** (220 mg, 0.60 mmol), Et_3N (0.50 mL, 3.6 mmol), CH_2Cl_2 (10 mL), and a crystal of 4-(dimethylamino)pyridine. After 1 h, the reaction was partitioned between Et_2O (50 mL) and saturated aqueous NH_4Cl (10 mL). The layers were separated, the organic layer was washed with brine (10 mL), dried ($MgSO_4$), and filtered, and the filtrate was concentrated. The residue was purified on silica gel (4:1 hexanes-EtOAc) to provide 150 mg (36%) of **24** as a colorless solid: mp 175-176°C: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, J = 7.8 Hz, 2H) 7.88 (d, J = 7.8 Hz, 2H) 7.56 (d, J = 7.8 Hz, 2H) 7.37-7.40 (m, 5H) 7.31 (d, J = 7.8 Hz, 2H) 5.15-5.25 (m, 2H) 4.79-4.82 (m, 1H) 4.52-4.53 (m, 2H) 4.41-4.45 (m, 1H) 2.56-2.61 (m, 1H) 2.48-2.53 (m, 1H) 2.31 (s, 3H) 2.13-

2.19 (m, 1H) 1.92-1.96 (m, 1H) 1.56-1.73 (m, 2H), the NH signal was too broad to observe; ^{13}C NMR (125 MHz, $CDCl_3$) 176.9, 165.7, 165.4, 152.7, 143.7, 136.8, 135.8, 131.8, 131.0, 131.0,130.6, 128.9, 128.6, 128.3, 128.3, 128.2, 126.4, 101.0, 66.1, 62.3, 56.0, 55.9, 34.7, 33.7, 27.4, 18.9 ppm; IR (film) 1716, 1608 cm^{-1}; $[\alpha]^{23}_{D}$ -3.3, $[\alpha]^{23}_{577}$ -2.8, $[\alpha]^{23}_{546}$ -1.0, $[\alpha]^{23}_{435}$ +32.5, $[\alpha]^{23}_{405}$ +68.5, (*c* 1.75, $CHCl_3$). Anal. Calcd for $C_{32}H_{29}Br_2N_3O_5$: C, 55.27; H, 4.20; N, 6.04. Found: C, 55.20; H, 4.16; N, 6.04.

(S)-N-[(Aminomethylene)-4-methoxy-2,3,6-trimethylbenzenesulfonamide]-3-amino-7-methyl-6-octenol (25a). A solution of (*S*)-3-amino-7-methyl-6-octenol (Overman et al., *J. M. Chem. Soc.* 1995. 117:2657) (**19**, 1.00 g, 6.36 mmol), *S,S*,-dimethyl *N*-(4-methoxy-2,3,6-trimethylbenzenesulfonyl)-carbonimidodithioate (1.78 g, 5.34 mmol), and benzene (6 mL) was maintained at reflux for 2 h. The reaction was quenched by adding Et_2O (50 mL) and 0.1 M HCl (5 mL). The layers were separated, the organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated. The resulting crude oil was purified by MPLC (1:1 hexanes-EtOAc) to provide 1.81 g (77%) of the corresponding pseudothiourea as a colorless oil: ^1H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, J = 9.8 Hz, 1H) 6.40 (s, 1H) 5.04-5.06 (m, 1H) 3.85 (s, 3H) 3.77-3.84 (m, 1H) 3.66-3.73 (m, 2H) 2.72 (s, 3H) 2.64 (s, 3H) 2.36 (s, 3H) 2.15 (s, 3H) 1.96-2.02 (m, 2H) 1.84-1.92 (m, 2H) 1.69 (m, 3H) 1.60-1.68 (m, 2H) 1.56 (m, 3H), the OH signal was too broad to observe; ^{13}C NMR (125 MHz, $CDCl_3$) 167.4, 158.8, 138.8, 137.0, 132.8, 132.4, 124.9, 122.7, 111.6, 58.7, 55.4, 52.2, 37.7, 35.4, 25.7, 24.1, 24.0, 18.4, 17.6, 14.2, 11.8 ppm; IR (film) 3480, 3290 cm^{-1}; $[\alpha]^{23}_{D}$ -15.3, $[\alpha]^{23}_{577}$ -14.7, $[\alpha]^{23}_{546}$ -17.9, $[\alpha]^{23}_{435}$ -31.8, $[\alpha]^{23}_{405}$ -39.2 (*c* 1.9, $CHCl_3$). Anal. Calcd for $C_{21}H_{34}N_2O_4S_2$: C, 56.98; H, 7.74; N, 6.33. Found: C, 56.90; H, 7.69; N, 6.34.

Silver nitrate (26 mL, 0.2 M in MeCN) was added dropwise to a 0°C solution of a 1.59 g (3.60 mmol) portion of this pseudothiourea and MeCN (75 mL) that had been saturated with NH_3. (Burgess et al., *J. Org Chem.*1994, 59:2179). The reaction mixture was allowed to warm to room temperature, and after 18 h, EtOAc (100 mL) was added and the resulting mixture was filtered through a plug of Celite. The eluent was concentrated to provide 1.46 g (99%) of **25a** as a colorless solid: mp 107-109°C: ^1H NMR (500 MHz, $CDCl_3$) δ 6.51 (s, 2H)

6.15 (s, 1H) 4.90 (app s, 1H) 4.36 (s, 1H) 3.80 (app s, 4H) 3.53-3.66 (m, 3H) 2.64 (s, 3H) 2.56 (s, 3H) 2.10 (s, 3H) 1.85-1.86 (m, 2H) 1.71 (m, 1H) 1.56 (m, 3H) 1.39-1.32 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) 158.5, 156.8, 138.3, 136.6, 132.9, 131.9, 124.8, 123.2, 111.7, 58.1, 55.3, 47.5, 38.4, 35.3, 25.5, 24.5, 24.1, 18.2, 17.4, 11.9 ppm; IR (film) 3442, 3354, 1621 cm^{-1}; $[\alpha]^{23}_D$ -20.0, $[\alpha]^{23}_{577}$ -20.7, $[\alpha]^{23}_{546}$ -23.0, $[\alpha]^{23}_{435}$ -33.2, $[\alpha]^{23}_{405}$ -35.7 (*c* 2.4, $CHCl_3$). Anal. Calcd for $C_{20}H_{33}N_3O_4S$: C, 58.37; H, 8.08; N, 10.21. Found: C, 58.31; H, 8.05; N, 10.21.

(S)-N-[(Aminomethylene)-4-methoxy-2,3,6-trimethylbenzenesulfonamide]-3-amino-1-benzyloxy-7-methyl-6-octene (25b). Following the procedure described for preparing **25a**, **13** (0.807 g, 3.262 mmol) was converted in 80% overall yield to **25b** a colorless oil; ^1H NMR 500 MHz, DMSO, 80°C) δ 7.25-7.32 (m, 5H) 6.65 (s, 1H) 6.45 (s, 1H) 6.42 (s, 1H) 5.01 (m, 1H) 4.35 (s, 2H) 3.77 (s, 3H) 3.73 (m, 1H) 3.38-3.41 (m, 2H) 3.09 (s, 3H) 2.63 (s, 3H) 2.56 (s, 3H) 1.88 (m, 2H) 1.69 (m, 1H) 1.60 (m, 4H) 1.49 (s, 3H) 1.36-1.42 (m, 2H); ^{13}C NMR (125 MHz, DMSO, 80°C) 157.3, 155.6, 138.2, 137.2, 135.3, 134.8,130.5, 127.7, 126.9, 126.8, 123.4, 123.2, 111.6, 71.6, 66.5, 55.1, 47.5, 34.3, 34.2, 24.8, 23.5, 22.8, 17.4, 16.9, 11.2 ppm; IR (film) 3445, 3336, 1622, 1538 cm^{-1}; $[\alpha]^{23}_D$ +14.6, $[\alpha]^{23}_{577}$ +15.3, $[\alpha]^{23}_{546}$ +18.2, $[\alpha]^{23}_{435}$ +37.4, $[\alpha]^{23}_{405}$ +48.9 (*c* 1.80, $CHCl_3$). Anal. Calcd for $C_{27}H_{39}N_3O_4S$: C, 64.64; H, 7.84; N, 8.38. Found: C, 64.77; H, 7.88; N, 8.32.

Conversion of 25a to 27c and 28c under Knoevenagel Biginelli Conditions. Following the represenative olefin dihydroxylation and 1,2-diol cleavage procedure, **25a** (100 mg, 0.24 mmol) was converted to **26a**. Aminal **26a** was then condensed with **16** following the representative procedure for Biginelli condensation under Knoevenagel conditions with the exception that the concentration of **26a** in 2,2,2-trifluoroethanol was 0.5 M. Purification of the crude product on silica gel (1:1 hexanes-EtOAc) provided 80 mg (61%) of a 6:1 mixture of **27a** and **28a**.

A 120 mg (0.22 mmol) sample of a comparable product was esterified with 4-bromobenzoyl chloride (160 mg, 0.72 mmol) following the procedure described for the preparation of **24** to provide a crude residue that was purified on silica gel (3:1 hexanes-EtOAc) to provide 160

mg (100%) of a 6:1 mixture **27c** and **28c**. These isomers were separated by HPLC (6:1 hexanes-EtOAc; 20 mL/min, 300 x 22 mm 10 μm silica Alltech column) to give pure samples of **27c** (t_R = 62 min) and **28c** (t_R = 53 min).

(4aR,7S)-7-[2-(4-Bromobenzoyloxy)ethyl]-1-(4-methoxy-2,3,6-trimethylbenzenesulfonylimino)-3-methyl-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxylic acid benzyl ester (27c): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (s, 1H) 7.76 (d, J= 8.4 Hz, 2H) 7.51 (d, J= 8.4 Hz, 2H) 7.32-7.39 (m, 5H) 6.48 (s, 1H) 5.12-5.21 (m, 2H) 4.20-4.29 (m, 2H) 4.13-4.18 (m, 1H) 4.05-4.09 (m, 1H) 3.78 (s, 3H) 2.66 (s, 3H) 2.59 (s, 3H) 2.46-2.55 (m, 1H) 2.34 (s, 3H) 2.13-2.19 (m, 1H) 2.06 (s, 3H) 1.93-2.00 (m, 1H) 1.75-1.87 (m, 2H) 1.64-1.71 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) 165.5, 164.9, 158.5, 148.1, 145.6, 138.4, 136.5, 135.6, 132.9, 131.6, 131.0, 128.7, 128.6, 128.4, 128.3, 128.0, 124.7, 111.6, 103.8, 66.3, 62.4, 57.0, 55.3, 54.9, 32.5, 30.0, 28.5, 24.1, 18.5, 18.3, 11.8 ppm; IR (film) 3292, 1716, 1614 cm^{-1}; $[\alpha]^{23}_D$ +55.5, $[\alpha]^{23}_{577}$ +57.7, $[\alpha]^{23}_{546}$ +66.5, $[\alpha]^{23}_{435}$ +121, $[\alpha]^{23}_{405}$ +150 (*c* 2.1, $CHCl_3$). Anal. Calcd for $C_{35}H_{38}BrN_3O_7S$: C, 58.01; H, 5.29; N, 5.80. Found: C, 57.98; H, 5.42; N, 5.52.

(4aS,7S)-7-[2-(4-Bromobenzoyloxy)ethyl]-1-(4-methoxy-2,3,6-trimethylbenzenesulfonylimino)-3-methyl-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxylic acid benzyl ester (28c): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (s, 1H) 7.76 (d, J = 8.4 Hz, 2H) 7.51 (d, J= 8.4 Hz, 2H) 7.33-7.54 (m, 5H) 6.44 (s, 1H) 5.12-5.23 (m, 2H) 4.36-4.44 (m, 2H) 4.27-4.29 (m, 2H) 3.80 (s, 3H) 2.65 (s, 3H) 2.56 (s, 3H) 2.46-2.51 (m, 1H) 2.29 (s, 3H) 2.02-2.10 (m, 4H) 1.75-1.82 (m, 1H) 1.48-1.62 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 165.6, 165.3, 158.5, 146.7, 143.0, 138.4, 136.9, 135.8, 133.0, 131.6, 131.0, 128.7, 128.6, 128.3, 128.2, 128.0, 124.8, 111.6, 100.6, 66.2, 62.0, 56.2, 56.1, 55.4, 34.5, 33.2, 27.7, 24.0, 18.9, 18.3, 11.8 ppm; IR (film) 3298, 1716, 1614 cm^{-1}; $[\alpha]^{23}_D$ -17.7, $[\alpha]^{23}_{577}$ -16.1, $[\alpha]^{23}_{546}$ -18.3, $[\alpha]^{23}_{435}$ -19.4, $[\alpha]^{23}_{405}$ -13.3 (*c* 0.75, $CHCl_3$). Anal. Calcd for $C_{35}H_{38}BrN_3O_7S$: C, 58.01; H, 5.29; N, 5.80. Found: C, 58.06; H, 5.41; N, 5.55.

Conversion of Compound 25b to 27b and 28b under Knoevenagel Biginelli Conditions. Following the represenative olefin dihydroxylation and 1,2-diol cleavage procedure, **25b** (100 mg, 0.20 mmol) was converted to **26b**, and this crude material was condensed with **16** following the representative procedure for Biginelli condensation under Knoevenagel conditions with the exception that the concentration of **26b** in 2,2,2-trifluoroethanol was 0.5 M. Purification of the crude product on silica gel (4:1 hexanes-EtOAc to 2:1 hexanes-EtOAc) provided 106 mg (84%) of a 7:1 mixture of **27b** and **28b**. Characterization data for the major product (4a*R*,7*S*)-7-(2-Benzyloxyethyl)-1-(4-methoxy-2,3,6-trimethylbenzenesulfonylimino 3-methyl-1,2,4a,5,6,7-hexahydropyrrolo[1,2-*c*]pyrimidine-4-carboxylic acid benzyl ester (**27b**) as determined from this mixture: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.42 (s, 1H) 7.23-7.42 (m, 10H) 6.52 (s, 1H) 5.15-5.25 (m, 2H) 4.28-4.36 (m, 2H) 4.23 (d, J = 11.1, 4.0 Hz, 1H) 4.03-4.07 (m, 1H) 3.82 (m, 3H) 3.40-3.42 (m, 2H) 2.70 (s, 3H) 2.62 (s, 3H) 2.48-2.50 (m, 1H) 2.31 (s, 3H) 2.13 (s, 3H) 2.00-2.05 (m, 1H) 1.93-1.95 (m, 2H) 1.79-1.83 (m, 1H) 1.47-1.53 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) 165.1, 158.5, 148.1, 145.6, 138.5, 138.2, 136.4, 135.7, 133.2, 128.6, 128.4, 128.2, 128.2, 127.5, 127.4, 124.7, 111.7, 103.9, 72.5, 67.7, 66.3, 57.0, 55.9, 55.3, 33.4, 30.0, 28.8, 24.0, 18.5, 18.3, 11.9 ppm; IR (film) 3289, 1704, 1614 cm^{-1}. Anal. Calcd for $C_{35}H_{41}N_3O_6S$: C, 66.54; H, 6.54; N, 6.65. Found: C, 66.66; H, 6.57; N, 6.66.

Conversion of Compound 25b to (4aS,7S)-7-(2-Benzyloxyethyl)-1-(4-methoxy-2,3,6-trimethylbenzenesulfonylimino)-3-methyl-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxylic Acid Benzyl Ester (28b) by Biginelli Condensation in the Presence of PPE. Following the representative procedure for olefin dihydroxylation and 1,2-diol cleavage, **25b** (100 mg, 0.20 mmol) was converted to **26b**. Crude aminal **26b** was then condensed with **16** following the representative procedure for Biginelli condensation in the presence of PPE to give, after purification on silica gel (2:1 hexanes-EtOAc to 1:1 hexanes-EtOAc), 77 mg (61%) of **28b**, which was contaminated with a trace of **27b** (3%). **28b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (s, 1H) 7.22- 7.42 (m, 10H) 6.54 (s, 1H) 5.16-5.26 (m, 2H) 4.36-4.40 (m, 2H) 4.26-4.35 (m, 2H) 3.84 (m, 3H) 3.45-3.48 (m, 2H) 2.72 (s, 3H) 2.65 (s, 3H) 2.45-2.50 (m, 1H) 2.32 (s, 3H) 2.15-2.20 (m, 1H) 2.14 (s, 3H) 2.00-2.05 (m, 1H) 1.62-1.72 (m, 1H) 1.51-1.60 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4, 158.5, 146.4, 142.9, 138.6, 136.4, 135.8,

133.4, 128.6, 128.3, 128.2, 128.2, 127.5, 127.4, 127.4, 124.7, 111.6, 100.5, 72.5, 67.2, 66.1, 56.4, 56.0, 55.3, 34.5, 34.1, 27.7, 24.0, 18.8, 18.3, 11.9 ppm; IR (film) 3290, 1712, 1614 cm^{-1}; $[\alpha]^{23}_{D}$ -65.8, $[\alpha]^{23}_{577}$ -67.5, $[\alpha]^{23}_{546}$ -76.7, $[\alpha]^{23}_{435}$ -117, $[\alpha]^{23}_{405}$ -128 (*c* 1.1, $CHCl_3$). Anal. Calcd for $C_{35}H_{41}N_3O_6S$: C, 66.54; H, 6.54; N, 6.65. Found: C, 66.49; H, 6.51; N, 6.56.

Conversion of Compound 28c to Compound 24. A solution of **28c** (15 mg, 20 mmol) and TFA (2 mL) was maintained for 1 h at room temperature. The reaction was concentrated, and the resulting crude oil was used without purification. 4-Bromobenzoyl chloride (22 mg, 0.10 mmol) was added to a 0°C solution of this crude guanidine, Et_3N (0.15 mL, 1.08 mmol), CH_2Cl_2 (2 mL) and a crystal of 4-(dimethlyamino)-pyridine. After 1 h, the reaction was quenched to Et_2O (10mL) and saturated aqueous NH_4Cl (2 mL). The layers were separated, the organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated. The residue was purified on silica gel (4:1 hexanes-EtOAc) to provide 4 mg (29%) of **24** as a colorless solid.

S,S-Dimethyl N-(4-Methoxy-2,3,6-trimethylbenzenesulfonyl)carbonimidodithioate guanylating agent (Figure 6). Ammonia was bubbled through a solution of 4-methoxy-2,3,6-trimethylbenzenesulfonyl chloride (Fujino et al., Chem. Pharm. Bull., 1981, 29:2825) (10.3 g, 43.6 mmol) and CH_2Cl_2 (100 mL) at 0°C. After 30 min, acetone (0.5 L) was added, and the reaction mixture was filtered through a plug of silica gel and concentrated. The resulting solid was trituated with Et_2O to provide 9.18 g (92%) of 4-methoxy-2,3,6-trimethylbenzenesulfonamide as a colorless solid: mp 175-176°C; ^1H NMR (400 MHz, acetone-d_6) δ 6.75 (s, 1H) 6.36 (s, 2H) 3.86 (s, 3H) 2.63 (s, 3H) 2.58 (s, 3H) 2.05 (s, 3H); ^{13}C NMR (100 MHz, acetone-d^6) 159.7. 139.0, 138.0, 134.6, 125.3, 113.0, 56.2, 24.4, 18.5, 12.3 ppm; IR (KBr) 3385, 3279, 2983, 2942, 1582, 1560, 1486, 1309, 1148, 1113 cm-^{1}. Anal. Calcd. for $C_{10}H_{15}NO_3S$: C, 52.38; H, 6.59; N, 6.11. Found: C, 52.46; H, 6.55; N, 6.05.
A solution of 4-methoxy-2,3,6-trimethylbenzenesulfonamide (9.15 g, 39.9 mmol) and DMF (50 mL) was added to a mixture of NaH (4.11 g, 98.6 mmol, washed 3x with hexanes) and DMF (20 mL) at 0°C. The reaction was allowed to warm to room temperature and was stirred vigorously for 10 min before CS_2 (6.9 mL, 11 mmol) was added. After another 10

min, MeI (7.85 mL, 126 mmol) was added. After another 15 min, the reaction was poured into saturated aqueous NH_4Cl (200 mL) and extracted with $CHCl_3$ (3 x 0.5 L). The combined organic layers were dried ($MgSO_4$), filtered through a plug of silica gel and concentrated. The crude solid was trituated with MeOH to provide 11.1 g (84%) of *S,S*-dimethyl *N*-(4-methoxy-2,3,6-trimethylbenzenesulfonyl)carbonimidodithioate as a colorless solid: mp 175-176°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.56 (s, 1H) 3.84 (s, 3H) 2.71 (s, 3H) 2.57 (s, 3H) 2.52 (s, 6H) 2.13 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 182.3, 159.3, 139.2, 138.5, 130.3, 125.0, 111.7, 55.4, 23.9, 18.5, 16.3, 11.9 ppm; IR (film) 2969, 2930, 1552, 1476, 1386, 1307, 1146, 997, 925, 804 cm^{-1}. Anal. Calcd. for $C_{13}H_{19}NO_3S_3$: C, 46.82; H, 5.74; N, 4.20. Found: C, 46.82; H, 5.73; N, 4.22.

Results

Biginelli Condensations of Tethered Ureido Aldehydes

To pursue whether the free hydroxyl group in intermediate compound **1** might be influencing stereoselection, Biginelli condensations of this intermediate and benzyl ether derivative 15 were examined (Figure 4). Like compound 1, the benzyl ether congener was accessed from (R)-methyl-3-hydroxy-7-methyl-6-octenoate (**11**) (Kitamuram et al., Org. Synth., 1992, 71:1). Reduction of compound **11** with $LiAlH_4$ and selective monobenzylation of the resulting diol by reaction with excess NaH and benzyl bromide in DMF at –40 to –10°C furnished compound **12**. Mitsunobu inversion of alcohol 12 with HN_3, (Loibner et al., Helv. Chim. Acta, 1976, 59:2100) followed by reduction of the resulting azide and reaction of the resulting primary amine with trimethylsilyl isocyanate, provided urea compound **14b** in 32% overall yield from compound **11**.

In prior studies, the double bond of compound **14a** had been cleaved with ozone, using a dimethyl sulfide workup, to generate compound **1** (Overman et al., supra). A more reproducible procedure was to add 1.5 equiv of morpholinium acetate to the crude reaction mixture after reductive workup of the ozonide, but prior to concentration. Replacing

dimethyl sulfide with polymer-bound triphenylphosphine eliminated contamination with DMSO. Mass spectral data of the product compound **1a** generated in this fashion indicated incorporation of morpholine (with loss of H_2O) and showed the virtual absence of higher molecular weight oligomers.

Alternatively, compound **15** was generated by dihydroxylation of the corresponding alkene precursor, followed by cleavage of the derived 1,2-diol with $Pb\text{-}(Oac)_4$ (Zelle et al., J. Org. Chem., 1986, 51:5032). Aminals **1a** and **15** were never subjected to an aqueous workup or purification, but rather were used directly following removal of either the phosphine polymer or lead salts by filtration and concentration of the filtrate after adding morpholinium acetate. These intermediates are not simply a mixture of stereoisomers, but at least three components as judged by ^1H and ^{13}C NMR data; multiple signals are observed for many carbon atoms in the ^{13}C NMR spectra, while broad peaks are seen in the ^1H NMR spectra and no aldehyde signal is apparent.

Biginelli condensations of crude compound **15** or **1a** (generated from 1 equivalent of compound **14a** or **14b**) were carried out under identical conditions by reaction with 1.5 equivalents of β-ketoester **16** and 1.5 equivalents of morpholinium acetate at 60°C in 2,2,2-trifluoroethanol. These conditions provided the *cis*- and *trans*-1-oxohexahydropyrrolo[1,2-c]pyrimidines **17a** and **18a** in a 4:1 ratio (80% yield) and the corresponding benzyl ether analogues **17b** and **18b** in an identical 4:1 ratio (81% yield). The β-oxygen substituent of the side chain clearly plays no significant role. Trifluoroethanol was employed as the reaction solvent since earlier studies with related intermediates had shown that cis stereoselection under Knoevenagel conditions was optimal in this highly polar solvent. For example, stereoselection in the condensation of **1** and **16** was 2:1 when ethanol was employed. Products **17a** and **18a** did not interconvert upon resubmission to reaction conditions. Stereochemical assignments for the hexahydropyrrolo[1,2-c]pyrimidine products followed from diagnostic ^1H NMR signals of the angular methine hydrogens H4a and H7: **17a** (4.25 and 4.11 ppm) and **17b** (4.29 and 4.00 ppm) (Overman and Rabinowitz., J. Org. Chem., 1993, 58:3235).

In a recent investigation, Kappe reported (J. Org. Chem., 1997, 62:7201) that the mild dehydrating agent polyphosphate ester (PPE) (Cava et al., J. Org. Chem., 1969, 34:2665) was an excellent promoter of the classical three-component Biginelli condensation. Condensation of **15** with β-ketoester **16** at room temperature in a 1:1 mixture of PPE and CH_2Cl_2 provided Biginelli products **17b** and **18b** in 60% yield, with the trans isomer **18b** now predominating to the extent of 4:1. Identical to what was observed under Knoevenagel conditions, compounds **17b** and **18b** were recovered unchanged when resubmitted to the PPE reaction conditions for 48 h.

Biginelli Condensations of Tethered Guanyl Aldehydes

Although three-component condensations of guanidines, aldehydes, and β-ketoesters are known, this modification of the Biginelli condensation has not been widely explored. (Kappe, Tetrahedron, 1993, 49:6937). To examine the tethered variant, unsaturated guanidinium alcohol **21** was prepared from (S)-amino alcohol **19** (Overman et al., J. Am.Chem. Soc., 1995, 117:2657) by condensation with 1H-pyrazole-1-carboxamidine hydrochloride (**20**) (Figure 5, Bernatowicz et al., J. Org. Chem., 1992, 57:2497). Ozonolysis of **21** followed by workup with dimethyl sulfide and concentration provided **22**, which like its urea counterpart was a mixture of several components. When **22** was concentrated with 1.5 equiv of morpholinium acetate, FAB mass spectral data indicated incorporation of morpholine with loss of H_2O; higher molecular weight oligomers were not observed for either **22** (X = OH) or its morpholine adduct. Both intermediates performed identically in Biginelli condensations. Without purification, **22** was condensed with β-ketoester **16**, using Knoevenagel conditions identical to those employed in the urea series, to afford a single Biginelli adduct **23** in 42% overall yield from **19**. This product had the trans stereochemistry as rigorously established by single-crystal X-ray analysis of dibenzoyl derivative **24** (Coordinates for compound **24** have been deposited with Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.).

To pursue the origin of the stereochemical reversal in the urea and guanidine series, Biginelli condensations of tethered N-sulfonylguanidine aldehydes **26** were investigated as depicted in Figure 6. Since the pK_a of N-sulfonylguanidinium salts is typically ~1, the sulfonylguanidine substituent electronically resembles more closely a urea than a guanidine (Tatlor et al., J. Chem. Soc. Perkin Trans. 2, 1986, 1765; Yamamoto et al., in *Synthesis and Chemistry of Guanidine Derivatives*, Yamamoto and Kojima, Ed., Wiley, New York, 1991 (Vol. 2, pp 485-526). The statistically corrected pK_a of a monosubstituted guanidinium salt bearing an SO_2NH_2 substituent has been determined to be 1.83 in water. Using the linear free energy correlation developed by Tatlor et al., supra, the value for the corresponding SO_2Me-substituted guanidinium salt would be 0.2. Treatment of amino alcohol **19,** or the corresponding amino ether **13,** with S,S-dimethyl N-(4-methoxy-2,3,6-trimethylbenzenesulfonyl)-carbonimidodithioate, followed by aminolysis with NH_3 and $AgNO_3$, afforded the Mtr-protected guanidines **25** in good yield (Burgess et al., J. Org. Chem., 1994, 59:2179). Dihydroxylation of these intermediates, followed by diol cleavage, provided **26a** and **26b.** These intermediates were again not simple mixtures of stereoisomers; multiple signals were observed for many carbon atoms in the ^{13}C spectra, while ^{1}H spectra exhibited broad peaks and showed no apparent aldehyde signal.

Biginelli condensation of crude **26b** with β-keto ester **16** under Knoevenagel conditions identical to those employed with the other substrates proceeded in 84% yield to give the *cis*- and *trans*-1-iminohexahydropyrrolopyrimidines **27b** and **28b** in a 7:1 ratio. Nearly identical stereoselectivity was realized in the hydroxyethyl series. In dramatic contrast, when the condensation of **26b** and **16** was carried out with PPE, the *trans*-1-iminohexahydropyrorolopyrimidine **27b** predominated to the extent of 20:1. Sulfonylguanidine products **27b** and **28b** were recovered unchanged when resubmitted for 48h to either the Knoevenagel or PPE reaction conditions.

Stereochemical assignments were made by chemical correlation of **28a** with **24**. Acylation of the crude product mixture produced from Biginelli condensation of **26a** and **16** under Knoevenagel conditions with 4-bromobenzoyl chloride followed by separating the isomers by

HPLC provided pure samples of **27c** and **28c**. Exposure of the minor product **28c** to TFA at room temperature removed the Mtr group, and acylation of the resulting free guanidine with 4-bromobenzoyl chloride provided **24**.

These results demonstrate that stereoselection in tethered Biginelli condensations to form 1-oxo- and 1-iminohexahydrophyrrolo[1,2-c]pyrimidines varies substantially depending on reaction conditions and the nature of the group X (Figure 3). With substrates having urea and N-sulfonylguanidine functionality, cis stereoselection (4-7:1) is observed when the condensation is accomplished under Knoevenagel conditions, while trans stereoselection (4-20:1) is observed when the condensation is carried out in the presence of polyphosphatester (PPE). Under both conditions, stereoselectivity was highest in the N-sulfonylguanidine series. With a substrate having a basic guanidine unit, the *trans* product is formed exclusively under Knoevenagel conditions. Since the Knoevenagel conditions are notably mild (morpholinium acetate in CF_3CH_2OH at 60°C), this latter guanyl aldehyde route to *trans*-1-iminohexahydropyrrolo[1,2-c]pyrimidines will like be particularly useful for the synthesis of Crambescidin and Batzelladine alkaloids having the anti relationship of the hydrogens flanking the pyrrolidine nitrogen. In the Examples below, the first total synthesis of Isocrambescidin 800 using this approach is described.

The origin of stereoselectivity is unknown. Without wishing to be bound by any theory, the following hypothesis is proposed (Figure 7). Under Knoevenagel conditions, the stereochemistry-determining step in condensations of ureido or N-sulfonylimino aldehyde intermediates **29** could be cyclization of Knoevenagel adduct **31** to give **33**. If this reaction has a late transition state, the cis-2,5-disubstituted pyrrolidine should be formed preferentially (molecular mechanics calculations on the model N-acylamino-2,5-disubstituted pyrrolidines **36** show that the cis isomer is 1.9 kcal/mol more stable than the trans isomer. Calculations were done using the MM2 force field and the Monte Carlo search routing of MacroModel V3.5X. In contrast, in the guanyl aldehyde series, loss of HY from **29** to form the corresponding iminium ion **30** should be particularly favorable, since the nitrogen substituent in **30** is a weakly electron-withdrawing amidine group. If addition of the enol (or enamine)

derivative of **16** is controlled primarily by destabilizing interactions with the side chain, trans adduct **32** should be produced preferentially in what could be the stereochemistry-determining step. Alternatively, the stereochemistry-determining step could be [4 + 2]-cycloaddition of the enol (or enamine) or **30** from the face opposite the side chain, followed by loss of water (or morpholine). In accord with Kappe's recent investigations of the mechanism of the three component Biginelli reaction under classical acidic conditions (Kappe et al., supra), condensations of the ureido or N-sulfonylimino aldehyde intermediates **29** in the presence of polyphosphate ester (PPE) could also proceed by the iminium ion pathway to provide largely *trans*-1-oxo- and 1-iminohexahydropyrrolo[1,2-c]pyrimidines.

These results demonstrate that stereoselection in the tethered Biginelli condensations depicted in Figure 3 can be tuned to give either the *cis* or *trans* product. Under optimum conditions, the *trans* isomer can be obtained in high stereoselectivity (>20:1) and the *cis* isomer in moderate selectivity (4-7:1). Tethered Biginelli condensations can be extended to include guanyl aldehyde substrates that produce Biginelli products, which should prove particularly useful for preparing complex guanidines.

EXAMPLE II

Enantioselective Total Synthesis of Ptilomycalin A, Crambescidin 800 and Selected Congeners

Synthesis Plan. A molecular mechanics model of the methyl ester of the Ptilomycalin A/Crambescidin core is shown in Figure 2. The triazacenaphthalene ring system of these alkaloids is nearly planar with the seven- and six-membered cyclic ethers being oriented on one face. Since the two C-O bonds are axial (cis to the C10 and C13 angular hydrogens), it was surmised that the C8 and C15 spirocenters might assemble with the required stereochemistry if the proper *cis* stereochemistry of the central triazacenaphthalene unit were in place. Setting the cis stereorelationship of the angular hydrogens at C10 and C13 and relating the chirality of this unit to the C3 and C19 stereogenic centers of the oxepene and hydropyran rings proved to be the critical elements in evolving a stereocontrolled strategy for

preparing this class of guanidine alkaloids.

As illustrated in Figure 8, disconnection of the C8 aminal and retrosynthetic cleavage of the C15-O bond of **36** leads to the 1-oxohexahydropyrrolo[1,2-c]pyrimidine (X =O) and 1-iminohexahydropyrrolo[1,2-c]pyrimidine intermediates (X =NH_2) **37**. The 4-alkoxycarbonyl-3,4-dihydropyrimidin-2(1H)-one part structure of **37** suggested that this essential bicyclic intermediate might be prepared by a novel modification of the three-component Biginelli condensation, in which the urea and aldehyde components would be linked as depicted in **38**.

This analysis has the appeal of high convergence, since the left-hand three rings of **36** would derive from acyclic fragment **38**, while the right two rings and the ester side chain would be incorporated as the simple β-ketoester unit **39**.

Enantioselective Total Synthesis of Ptilomycalin A. In light of the difficulty experienced during degradation studies in removing the ester side chain of **1**, the 16-hydroxyhexadecanoic acid fragment was incorporated from the outset (Figure 9). Alkylation of the dianion of methyl acetoacetate (**44**)(Huckin and Weiler, J. Am. Chem. Soc., 1974, 96:1082) with enantiopure (R)-siloxy iodide **45** provided **46** in 73% yield. Iodide **45** is conveniently available in high yield from methyl (R)-2-hydroxybutanoate (Kitamura et al., Org. Synth., 1992, 71:1). Selective transesterification (Taber et al., J. Org. Chem., 1985 50:3618) of the β-ketoester functionality with allyl 16-hydroxyhexadecanoate using DMAP (4-dimethylaminopyridine) as catalyst gave **47** in 64% overall yield from **44.**

Since the tethered Biginelli condensation had just been verified, in this first generation approach this critical reaction was selected as early as possible in the synthetic sequence. For this reason, a less convergent strategy was pursued in which the electrophilic component of the Biginelli condensation was simplified by deletion of the C1-C7 fragment. The precursor of this intermediate, urea **50**, was prepared in three steps from enantiopure methyl (R)-3-hydroxy-7-methyloct-6-enoate (**48**)(Kitamura et al., supra) as summarized in Figure 10.

Mitsunobu displacement of **48** with hydrazoic acid followed by reduction of the crude β-azido ester with $LiAlH_4$ gave *S* amino alcohol **49** in 72% yield and in >98% ee. Entantiometric excess was determined by evaluation of the ^{19}F NMR spectra of the corresponding (R)- and (S)- Mosher's amides. Use of other nitrogen nucleophiles such as phthalimide in the Mitsunobu reaction led to significant amounts of the corresponding α, β-unsaturated ester.

Condensation of **49** with potassium cyanate and HCl under standard conditions provided unsaturated urea **50** in 82% yield after recrystallization. Ozonolysis **50** in MeOH at -78°C, followed by reduction of the intermediate hydroperoxide with Me_2S and concentration furnished a viscous yellow oil. Further concentration of this product at 0.1 Torr for 5 days at 50°C to remove residual Me_2SO lead to a nearly colorless amorphous powder. This intermediate is more complex than formulation **51** implies. Multiple signals were observed for many carbon atoms in the ^{13}C NMR spectra and the ^{1}H NMR spectrum was broad; no aldehyde signal was apparent, and mass spectral data indicated an oligomeric consistency. All attempts to enhance the purity of **51** by chromatography were unsuccessful.

Biginelli condensation of crude **51** and **47** under the conditions developed during our previous model study (Overman et al., J. Org. Chem., 1993, 58:3235-3237), proceeded in low yield. A number of reaction parameters were surveyed and reaction efficiency was improved in polar solvents. The best result was achieved by heating a mixture of crude **51**, 1.5 equivalent of β-ketoester **47**, 1 equivalent of morpholinium acetate, a catalytic amount of acetic acid and excess Na_2SO_4 at 70°C in EtOH. Purification of the resulting product on silica gel provided *cis* adduct **52** in 61% yield and *trans* adduct **53** in 8% yield. Stereochemical assignments for the hexahydropyrrolo[1,2-c]pyrimidine products followed from the similarity of their angular methine hydrogen signals (**52**: 4.25 and 4.11 ppm and **53**: 4.44 and 4.09 ppm) with those of **41** and its *trans* epimer, the latter of which had earlier been analyzed by single-crystal X-ray analysis (Overman et al., J. Org. Chem., 1993, 58:3235-3237). In a recent detailed examination of stereoselection in related Biginelli condensations (McDonald and Overman, J. Org. Chem., 1999, 64:1520-1528) a reproducible procedure for generating the electrophilic

reaction component and carrying out the Biginelli condensation was developed; these conditions reliably provide *cis* adducts in yields of 60-65%.

While **52** could be converted in one step to the spirotricyclic intermediate **54** by exposure to a slight excess of *p*-toluenesulfonic acid (*p*-TsOH), the reaction was more reproducible on a large scale if the TBDMS group was first cleaved with pyridinium *p*-toluenesulfonate (PPTS) in MeOH and the resulting alcohol cyclized at room temperature in $CHCl_3$ with a catalytic amount of *p*-TsOH (Figure 11). This sequence provided a single tricyclic product **54** in near quantitative yield. That this compound was epimeric to Ptilomycalin A at C14 was signaled by the 11.5 Hz diaxial coupling constant of the C14 methine hydrogen. (The Crambescidin numbering system is employed here.)

That high diastereoselectivity would be realized in forming the spirohydropyran was established in our earliest model study and can be rationalized as outlined in (Figure 12). Protonation of the vinylogous carbamate **57** to generate **58** followed by spirocyclization from the convex β-face would produce the spiroaminal having the oxygen axially disposed. Axial protonation of the exocyclic ketene hemiacetal, either prior or subsequent to spirocyclization, would deliver **54**.

Although epimerization of **55** to the axial ester might have been possible at this point, this adjustment was deferred to the final stage of the synthesis, hoping to benefit from a presumed thermodynamic preference for this group to be axial in the natural product. To prepare for the addition of the remaining carbons of the guanidine core, **54** was oxidized with the Swern reagent (Mancuso et al., J. Org. Chem. , 1978, 43:2480) to provide **55**, whose urea moiety was protected and activated for subsequent guanidine formation by *O*-methylation. It was critical that this methylation be performed under carefully optimized mild conditions, and that pseudourea **56** be purified rapidly on Et_3N-treated silica gel, or else significant epimerization at C10 resulted.

At this point the remaining C1-C7 carbons of the pentacyclic guanidine unit needed to be

appended. This elaboration proved to be extremely challenging. In early studies, we were unsuccessful in efficiently coupling lithium, cerium, titanium, or zirconium reagents derived from bromide **61** (Figure 13) to the benzyl ester congener of **56**. A complicating issue was the rapid epimerization of **56** at C10 in the presence of Lewis acidic reagents. The Grignard reagent derived from **61** added in acceptable yield to **56** at -78°C. Quenching this reaction at low temperature with morpholinium acetate and immediate filtration to remove magnesium salts provided the corresponding adduct as a mixture of alcohol epimers. Direct oxidation of this intermediate under Swern conditions (Mancuso et al., J. Org. Chem., 1978, 43:2480) provided **62** in 58% yield from **56**. Approximately 5% of a diastereomer, resulting from the minor enantiomer of **61**, was removed at this point. Bromide **61** was originally prepared in 86% ee by an asymmetric reduction of an ynone precursor (Overman et al., J. Am. Chem. Soc., 1995, 117:2657-2658). This sequence was extremely sensitive and yields were markedly eroded if magnesium salts resulting from the Grignard step were not removed quickly and thoroughly.

Cleavage of the silyl protecting group of **62** with TBAF furnished alcohol **63** which was then treated with ammonia and ammonium acetate, under conditions similar to those originally reported by Snider (Snider and Shi, J. Am. Chem. Soc., 1994, 116:549-557). After purification of the crude product on silica gel using an eluent containing formic acid, **64** was isolated in 60% as its formate salt (^{1}H NMR δ 8.23, ^{13}C NMR δ 165.8). Only a single pentacyclic guanidine was detected, with formation of the spiroaminal again occurring preferentially by axial C-O bond formation. A model of a tetracyclic cation **69** that is the likely direct precursor of the pentacyclic guanidine is shown in Figure 14; the C1-C7 side chain was replaced with a methyl group in generating this molecular mechanics model. A torsional preference for axial addition to the electron-deficient carbon is apparent in this figure and may be responsible for the high selectivity realized.

The total synthesis of Ptilomycalin A was readily completed from **64**. The allyl ester of this intermediate was cleanly cleaved (Deziel, Tetrahedron Lett., 1987, 28:4371), using palladium (0) catalysis and the resulting acid was coupled with the bis-BOC-protected spermidine **65**

(Cohen, et al., Chem. Soc., Chem. Commun., 1992, 298) to generate amide **66** (Figure 13). The ester was then epimerized by heating in MeOH in the presence of excess Et_3N, however, equilibrium for this epimerization favored the β epimer to the extent of 2-3:1. As a result, three recycles were required to obtain α-ester **67** in 50% yield. The equatorial C14 methine hydrogen of **67** showed a diagnostic doublet (J = 4.8 Hz) at δ 2.93. Finally, cleavage of the BOC protecting groups with HCO_2H, followed by concentration and washing with aqueous NaOH-NaCl provided (-)-Ptilomycalin A trihydrochloride (**1**) in high yield. Synthetic compound **1** showed ^1H and ^{13}C NMR spectra consistent with those reported for (-)-Ptilomycalin A (Kashman et al., J. Am. Chem. Soc., 1989, 111:8925-8926; Ohtani et al., J. Am. Chem. Soc., 1992, 114:8472-8479) and was indistinguishable from an authentic sample by TLC comparisons on three adsorbents. Synthetic **1** was converted to derivative compound **68**, which also exhibited ^1H and ^{13}C NMR spectra indistinguishable from those reported (Ohtani et al., supra). Synthetic compound **68** showed $[\alpha]^{23}_D$ -15.9 (*c* 0.8, $CHCl_3$), nearly identical to the rotation, $[\alpha]^{23}_D$ -15.8 (*c* 0.7, $CHCl_3$), reported for this well-characterized derivative of the natural product (Ohtani et al., supra).

Second Generation Synthesis Plan. A second generation synthesis of the Ptilomycalin A/Crambescidin alkaloids was undertaken with two specific goals in mind; (1) to achieve the high level of convergence originally illustrated in Figure 8, where the entire carbon skeleton of pentacycle **36** derive from a Biginelli condensation between a fully elaborated electrophilic component (**38**) and β-keto ester unit **39**; and (2) to gain access to either *cis* or *trans* **37** from a common precursor, thereby providing a convenient route to both the Crambescidin and Isocrambescidin core from a common intermediate. Details of the total synthesis of 13,14,15-Isocrambescidin 800 (**6**) are described in the following Example. Critical to both these syntheses is the rapid and stereoselective construction of the common C1-C13 fragment (amine precursor to urea **38**). This goal could be accomplished by combining the C1-C7 fragment **56** with the C8-C13 fragment **48** prior to the Biginelli condensation.

EXAMPLE III

Synthesis of Crambescidin 800 (Compound 2). The synthesis of the C1-C13 fragment began with conversion of 3-butynol (compound **70**) to the *p*-methoxybenzyl (PMB) ether **71** (Figure 15). The alkyne of **71** was deprotonated with *n*-buthyllithium at –40°C and the resulting acetylide treated with anhydrous DMF to provide ynal **72** in 90% yield, after quenching the intermediate α-aminoalkoxide into aqueous phosphate buffer (Journet et al., Tetrahedron Lett., 1988, 39:6427). The C3 stereocenter was introduced by the method of Weber and Seebach (Singh et al., J. Am. Chem. Soc., 987, 109:6187) through condensation of ynal **72** with Et_2Zn in the presence of (-)-TADDOL (20 mol%) and $Ti(Oi\text{-}Pr)_4$ to give (S)-**73** in 94% yield and >98% ee. This asymmetric transformation was reliably performed on a 45 g scale. Propargylic alcohol **73** was protected as the triisopropylsilyl (TIPS) ether and the alkyne partially hydrogenated with Lindlar's catalyst to provide *cis* alkene **74**. The PMB protecting group was oxidatively removed with DDQ and the resulting alcohol converted to iodide **75** in an overall yield of 89% from **73**.

Enantiopure methyl (R)-3-hydroxy-7-methyloct-6-enoate (compound **48**) (Kitamura et al., Org. Synth., 1992,71:1) was converted to amide in 88% yield by reaction with *N,O*-dimethylhydroxylamine hydrochloride according to the procedure of Weinreb (Garigipati et al, J. Am. Chem. Soc., 1985, 107:7790) followed by protection of the secondary alcohol as the triethylsilyl (TES) ether (Figure 16). Iodide **75** was converted to the corresponding lithium reagent and coupled with **76** to generate dienone **77** in 60-70% yield. Masking the C8 carbonyl of **77** as the ketal was necessary to prevent dehydration, which occurred under the Mitsunobu conditions employed to install the β-amino functionality. Ketalization was sluggish, however, when the β-hydroxy group was protected, so optimized reaction conditions were found which cleaved the TES group, did not promote β-hydroxy elimination of the intermediate β-hydroxy ketone and promoted ketalization. The novel ketalization conditions developed involved treatment of **77** with orthoester **78** (Roush and Gillis, J. Org. Chem., 1980, 45:4283-4287; Baganz and Domaschke, Chem. Ber., 1958, 91:650-653) and 1,3-propanediol in the presence of Amberlyst-15 to provide ketal **79** in 80%

yield. Mitsunobu displacement of the secondary alcohol with azide followed by reduction to the amine provided compound **80** in 77% yield from compound **79**. Amine **80**, synthesized in 11 steps and in ~30% overall yield from commercially available 3-butynol, was to serve as the common C1-C13 fragment for both the Crambescidin and Isocrambescidin syntheses (See Figure 16).

Condensation of amine **80** with TMSNCO yielded urea **81** in 89% yield (Figure 17). Selective dihydroxylation of the trisubstituted double bond of **81** (Sharpless and Williams, Tetrahedron Lett., 1975, 3045-3046) followed by cleavage of the vicinal diol with $Pb(OAc)_4$ in toluene and addition of morpholinium acetate yielded intermediate **82**, which was used without purification. Biginelli condensation of crude **82** with β-ketoester **47** under optimal Knoevenagel conditions (McDonald and Overman, J. Org. Chem., 1999, 64:1520-1528) provided an inseparable 6-7:1 mixture of desired *cis* adduct **83** and undesired *trans* adduct **84** in 61% overall yield from urea **81**. Stereochemical assignments for the hexahydropyrrolo[1,2-*c*]pyrimidine products followed from the similarity of their H13 angular methine hydrogen signals (**83**: 4.22 ppm and **84**: 4.44 ppm) with those of **41** and its *trans* epimer and **52** and **53** (Figure 17).

The silyl protecting groups of **83** were next discharged with TBAF to provide the corresponding urea diol (Figure 18). Brief exposure of this crude diol to *p*-TsOH induced spirohydropyran formation and ketal deprotection, affording **85** in 71% for the two steps. After protection of the secondary alcohol of **85** as the chloroacetate the minor trans isomer (~12%) was easily separated from the desired *cis* isomer **86**, which was isolated in 86% yield. It was necessary to protect the C3 alcohol of **85** to prevent methyl ether formation during the methylation of the urea functionality. Exposure of urea **86** to excess MeOTf in the presence of a hindered pyridine base cleanly provided the corresponding methyl pseudourea, which was directly converted to the guanidine without intermediate silica gel purification. It was critical that the methyl pseudourea not be exposed to silica gel chromatography, as decomposition and epimerization at C10 resulted under typical purification conditions. The ability to transform the urea functionality to the guanidine without manipulations of the intermediate

pseudourea represents one of the major advantages of the second generation synthesis over the first. After considerable experimentation, we found that optimal guanylation/cyclization conditions, saturated NH_3 in allyl alcohol buffered with NH_4Cl at 60°C for 1 day, cleanly provided pentacycles **87** and **88** in 81% from **86** as a 1.5:1 diastereomeric mixture at C14. Subjection of pure compound **88** to the reaction conditions established this ratio as the thermodynamic equilibrium (Figure 18).

These reaction conditions represent a significant improvement from those used in the first generation synthesis, as yield improved dramatically, and both deprotection of the C3 protecting group and the desired epimerization of C14 to its thermodynamic ratio occurred. The chloride counter ion was also obtained directly, eliminating detrimental aqueous washes. It should be noted that incomplete deprotection of the C3 alcohol occurred when a simple acetyl protecting group was employed, whereas the chloroacetyl protecting group was quantitatively removed under the guanylation/pentacyclization reaction conditions. Allyl alcohol was employed as solvent to avoid transesterification of the allyl ester that occurred when ethanol or methanol was employed. Furthermore, it was found necessary to saturate the reaction solution with NH_3 at 0°C prior to heating in order to achieve thermodynamic equilibration of the C14 ester side chain. Unfortunately, the thermodynamic ratio of 1.5:1 favored the undesired β-epimer (H14: J = 11.5 Hz). Pentacycles **87** and **88** were separated by medium pressure silica gel liquid chromatography, and the β-epimer twice recycled through the guanylation/cyclization conditions to provide the major α-ester pentacycle **88** in a 52% overall yield from tricyclic urea **86**.

The synthesis of Crambescidin 800 (compound **2**) was completed as follows (Figure 19). After removal of the allyl protecting group of **88** with $Pd(PPh_3)_4$ and morpholine (Deziel, supra) acid **89** was coupled with (*S*)-7-hydroxyspermidine **90** using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)(Castro et al. Tetrahedron Lett. 1975 1219-1222) to provide the corresponding amide **91** in 82% yield. Removal of the BOC groups with 3 M HCl in ethyl acetate (Stahl et al., J. Org. Chem., 1978 43:2285-2286) followed by purification of the crude product using reverse-phase HPLC

provided the trihydrochloride salt of Crambescidin 800 (**2**) in 75% yield. The data for the trihydrochloride salt of synthetic **2** is in agreement with the ^{1}H and ^{13}C NMR data reported for natural compound **2** (Jares-Erijman et al., J. Org. Chem., 1991, 56:5712-5715; Berlinck et al., J. Nat. Prod., 1993, 56:1007-1015). Synthetic **2** was also converted to the triacetate derivative, **92**. Data for synthetic **92** is also in agreement with ^{1}H and ^{13}C NMR data reported for **92** prepared from natural **2** (Id.) The Mosher's derivatives of (43*S*)- and (43*R*)-crambescidin 800 (**93**) were made and compared to the corresponding Mosher's derivative prepared from ~150 μg of natural compound **2**. The ^{19}F NMR data is identical for the Mosher's derivative prepared from natural **2** and synthetic **2**, thereby for the first time unambiguously establishing that the C43 stereochemistry of Crambescidin 800 is *S* (Figure 19).

Conclusion. The first total synthesis of Crambescidin 800 (**2**) was accomplished in a convergent fashion with the longest linear sequence from commercially available starting material being 25 steps and in a 3.0% overall yield. This synthesis demonstrates for the first time that the tethered Biginelli condensation can be accomplished under suitable mild conditions that the aldehyde-urea fragment can contain all the atoms of the three left rings (C1-C13), thus allowing high convergence and efficiency in the synthesis of Crambescidin/Ptilomycalin A alkaloids. These investigations confirm the stereochemical assignment of **2** and rigorously establish that the absolute configuration of its hydroxyspermidine side chain is *S*.

Experimental Section

General. Dry THF, Et_2O, and CH_2Cl_2 from Aldrich were filtered through a column charged with Al_2O_3 (solvent purification system). Triethylamine (Et_3N), pyridine, diisopropylethylamine (i-Pr_2NEt), diisopropylamine, and acetonitrile were distilled from CaH_2 at atmospheric pressure. Silica gel (0.040-0.063) by Merck was used for flash chromatography. The NMR spectra were recorded on the Bruker instruments (500 MHz and 400 MHz). The IR spectra were measured on Perkin-Elmer Series 1600 FTIR, and optical rotations were measured on Jasco DIP-360 polarimeter. Mass spectra were measured on a

MicroMass Analytical 7070E (CI-isobutane) or a MicroMass AutoSpec E (FAB) spectrometer. Infrared spectra were recorded using a Perkin Elmer 1600 FTIR spectrometer. Microanalyses were performed by Atlantic Microlabs, Atlanta, GA. Other general experimental details have been described (Metais et al., J. Org. Chem. 1997, 62:9210, incorporated by reference herein).

Synthesis of 1-(4-Methyoxybenzyloxy)-3-butyne (71). According to established procedures (Takaku et al., Tetrahedron lett., 1983, 24:5363; Nakajima et al., Tetrahedron Lett., 1988, 29:4139, both of which are incorporated by reference herein), TfOH (1.6 mL, 18 mmol) was added dropwise to a 0°C solution of PMBOC(=NH)CCl_3 (169.3 g, 0.6 mol), 3-butyn-1-ol (**70**) (67 g, 0.66 mol) and dry Et_2O (600 mL). After 30 min the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ (100 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (50 mL). The combined organic phases were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was diluted with hexanes (300 mL), filtered through a plug of silica gel, concentrated and stirred under vacuum (0.1 mm Hg) at 50°C for 12 h, yielding 114 g (~100%) of **71**, which was used without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, J = 8.4 Hz, 2 H), 6.89 (d, J = 8.4 Hz, 2 H), 4.49 (s, 2 H), 3.80 (s, 3 H), 3.58 (t, J = 7.0 Hz, 2 H), 2.49 (dt, J = 7.0, 2.7 Hz, 2 H), 2.00 (t, J = 2.6 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.2, 130.0, 129.3, 113.7, 81.3, 72.5, 69.2, 67.8, 55.2, 19.8 ppm; IR (film) 3292, 3001, 2936, 2863, 1614, 1514, 823 cm^{-1}; Anal. Calcd for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42. Found: C, 75.60; H, 7.49.

Synthesis of 5-(4-Methoxybenzyloxy)-2-pentynal (72). According to established procedures (Journet et al., supra) a hexane solution of *n*-BuLi (2.5 M, 32 mL) was added dropwise to a -40°C solution of **71** (14.45 g, 76.22 mmol) in dry THF (0.2 L). The reaction temperature did not exceed -35°C. After 10 min anhydrous DMF (11.8 mL, 153 mmol) was added in one portion and the cold bath was removed. After 30 min the reaction mixture was quenched by pouring into a vigorously stirred and cooled (~5°C) solution of 10% aqueous KH_2PO_4 (0.4 L) and methyl tert-butyl ether (MTBE) (0.38 L). After 20 min the layers were separated and the organic layer was washed with H_2O (50 mL). The combined aqueous layers were back

extracted with MTBE (100 mL), and the combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$), filtered and the filtrate concentrated. Purification of the residue on silica gel (10:1 hexanes-EtOAc; 6:1 hexanes-EtOAc) provided 14.97 g (90%) of **72** as a slightly yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.16 (s, 1 H), 7.26 (d, J = 8.5 Hz, 2 H), 6.88 (d, J = 8.6 Hz, 2 H), 4.48 (s, 2 H), 3.79 (s, 3 H), 3.61 (t, J = 6.7 Hz, 2 H), 2.69 (t, J = 6.7 Hz, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.0, 159.2, 129.6, 129.3, 113.8, 95.7, 81.9, 72.7, 66.5, 55.2, 20.6 ppm; IR (film) 3002, 2865, 2205, 1668, 1514, 824 cm^{-1}; Anal. Calcd for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.42; H, 6.54.

Synthesis of (5S)-Hydroxy-1-(4-methoxybenzyloxy)-3-heptyne (73). According to the general procedure of Seebach (Webber and Seebach, Tetrahedron 1994, 50:7473-7484), incorporated by reference herein, Ti(O*i*-Pr)$_4$ (12.2 mL, 41.0 mmol) was added to a 23°C solution of (4*R*, 5*R*)-2,2-dimethyl-α,α,α'α'-tetra(naphth-2-yl)-1,3-dioxolan-4,5-dimethanol (27.3 g, 41.0 mmol) and dry toluene (340 mL). After 3 h, solvent was removed under reduced pressure (0.1 mm). The resulting residue was dissolved in dry Et $_2$O (560 mL) and the reaction vessel was cooled to -50°C, whereupon Ti(O*i*-Pr)$_4$ (70 mL, 0.24 mmol) and **72** (44.7 g, 0.20 mmol) were added. Diethyl zinc (243 mL, 267 mmol, 1.1 M solution in toluene) was then added slowly over 1 h. The reaction vessel was then warmed to –27°C. After 18 h the reaction mixture was quenched with saturated aqueous NH_4Cl (100 mL). The organic phase was dried ($MgSO_4$), filtered through Celite® and concentrated. The resulting residue was purified on silica gel (20:1 hexanes-EtOAc; 5.6:1 hexanes-EtOAc; 1:1 hexanes-EtOAc) to provide 47.6 g (94%) of **73** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, J = 8.4 Hz, 2 H), 6.86 (d, J = 8.4 Hz, 2 H), 4.46 (s, 2 H), 4.26 (t, J = 6.4 Hz, 1 H), 3.78 (s, 3 H), 3.53 (t, J = 7.0 Hz, 2 H), 2.58 (s, 1 H), 2.49 (dt, J = 7.0, 1.5 Hz, 2 H), 1.66 (m, 2 H), 0.97 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1, 129.9, 129.2, 113.7, 82.3, 81.7, 72.4, 67.9, 63.5, 55.1, 30.9, 19.9, 9.4 ppm; IR (film) 3418, 2965, 1613, 1514, 1249, 823, 733 cm^{-1}; Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.26; H, 8.14. $[\alpha]^{25}_D$ -3.2, $[\alpha]^{25}_{577}$ -3.6, $[\alpha]^{25}_{545}$ -4.0, $[\alpha]^{25}_{435}$ -6.5, $[\alpha]^{25}_{405}$ -7.7, (*c* 2.35, $CHCl_3$).

Following the general procedure of Ward (Ward et al., Tetrahedron Lett., 1991, 32:7165-

7166), incorporated by reference herein, **73** (23 mg) was treated with (R)-α-methoxy-α-(triflouromethyl)phenylacetic acid chloride [(*R*)-MTPACl] to give the corresponding (*R*)-MTPA ester. Capillary GC analysis [150°C to 200°C/2.0°C min^{-1}, t_R 73-(*R*)-MTPA =3D 21.13 min, t_R ent-50-(R)-MTPA = 20.69 min] showed a ratio for 99.7:0.3 of 73-(*R*)-MTPA and *ent*-73-(*R*)-MTPA.

Synthesis of (S)-(Z)-1-(4-Methyloxybenzyloxy)-5-triisopropylsiloxy-3-heptene (74). Triisopropylsilyl trifluoromethanelsulfonate (19.1 mL, 71.1 mmol) was added dropwise over 15 min to a 0°C solution of 2,6-lutidine (10.3 mL, 88.4 mmol), 73 (14.6 g, 58.6 mmol) and dry CH_2Cl_2 (150 mL). After 1 h, the solution was poured into Et_2O (400 mL) and washed with 1N HCl (3 x 50 mL) and brine (20 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated. The crude oil was placed under vacuum (0.1 mm) overnight to provide 24.0 g (~100%) of (*S*)-1-(4-methoxybenzyloxy)-5-triisopropylsiloxy-3-heptyne as a slightly yellow oil, which was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ (d, *J* = 8.6 Hz, 2 H), 6.91 (d, *J* = 8.6 Hz, 2 H), 4.50 (s, 2 H), 4.24-4.45 (m, 1 H), 3.83 (s, 3 H), 3.59 (t, *J* = 7.2 Hz, 2 H), 2.54 (dt , *J* = 7.2, 1.9 Hz, 2 H), 1.67-1.76 (m, 2 H), 1.01-1.19 (m, 21 H), 1.02 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 159.1, 130.2, 129.2, 113.7, 82.9, 80.8, 72.5, 68.2, 64.3, 55.1, 32.1, 20.1, 18.0, 12.2, 9.4 ppm; IR (film) 2942, 2866, 1614, 1514, 1464, 1249, 1100 cm^{-1}; Anal. Calcd. for $C_{24}H_{40}O_3Si$: C, 71.24; H, 9.86. Found: C, 71.18; H, 10.04; $[\alpha]^{25}_D$ -25.5, $[\alpha]^{25}_{577}$ -26.3, $[\alpha]^{255}_{46}$ -30.5, $[\alpha]^{254}_{35}$ -50.8, $[\alpha]^{25}_{405}$ -60.8, (*c* 1.40, $CHCl_3$).

A mixture of crude (*S*)-1-(4-methoxybenzyloxy)-5-triisopropylsiloxy-3-heptyne (24.0 g, 58.6 mmol), freshly distilled quinoline (0.14 mL, 1.18 mmol), Lindlar's catalyst ($Pd/CaCO_3$ poisoned with PbO, 1.51 g) and dry 3:1 hexanes-EtOAc (360 mL) was maintained at 23°C under 1 atm H_2 for 17 h. This mixture was then filtered through a plug of Celite, and the eluent was concentrated to yield 24.0 g (~100%) of **74**, which was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30(d, *J* = 8.6 Hz, 2 H), 6.91 (d, *J* = 8.6 Hz, 2 H), 5.47-5.52 (m, 1 H), 5.37-5.43 (m, 1 H), 4.48-4.52 (m, 1 H), 4.48 (s, 2 H), 3.83 (s, 3 H), 3.46-3.50 (m, 2 H), 2.35-2.43 (m, 2 H), 1.59-1.68 (m, 1 H), 1.47-1.56 (m, 1 H), 1.09 (app s,

21 H), 0.89 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 159.1, 135.8, 130.4, 129.2, 124.4, 113.7, 72.6, 69.9, 69.4, 55.2, 31.6, 28.7, 18.0, 12.3, 9.3 ppm; IR (film) 2942, 2866, 1613, 1514, 1248, 1097 cm^{-1}; Anal. Calcd. for $C_{24}H_{42}O_3Si$: C, 70.88; H, 10.41. Found: C, 71.06; H, 10.44; $[\alpha]^{25}_D$ 18.5, $[\alpha]^{25}_{577}$ +19.7, $[\alpha]^{25}_{546}$ +22.6, $[\alpha]^{25}_{435}$ +41.9, $[\alpha]^{25}_{405}$ +52.0, (*c* 1.80, $CHCl_3$).

Synthesis of (S)-(Z)-1-Iodo-5-triisopropylsiloxy-3-heptene (75). A solution of crude 74 (24.0 g, 58.6 mmol), DDQ (17.3 g, 76.2 mmol) and 20:1 CH_2Cl_2 -H_2O (210 mL) was maintained at 23°C for 1 h. The reaction mixture was quenched by pouring into Et_2O (600 mL) and washing with 1N NaOH (2 x 200 mL) and brine (200 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated. Chromatagraphic separation of *p*-methoxybenzaldehyde was facilitated by reduction to *p*-methoxybenzyl alchohol. Towards this end, a solution of the resulting residue, MeOH (200 mL) and $NaBH_4$ (2.9 g, 77 mmol) was maintained at 23°C for 1 h. The reaction mixture was quenched by pouring into Et_2O (300 mL) and washing with 1N HCl (50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated. The resulting oil was purified on silica gel (20:1 hexanes-EtOAc; 15:1 hexanes-EtOAc; 10:1 hexanes:EtOAc) to provide 16.0 g (95%) of (*S*)-(*Z*)-5-triisopropylsiloxy-3-heptenol as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.55-5.51 (m, 1 H), 5.38-5.33 (m, 1 H), 4.47 (ddd, J = 13.5, 6.5, 1.5 Hz, 1 H), 3.66 (m, 2 H), 2.35-2.30 (m, 2 H), 1.65-1.57 (m, 1 H), 1.53-1.46 (m, 1 H), 1.41 (br s, 1 H), 1.05 (br s, 21 H), 0.87 (t, J = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) 137.0, 124.1, 69.9, 62.3, 31.7, 31.6, 18.0, 12.3, 9.3; IR (film) 3313, 2970, 2867; 1485, 1085, 1052 cm^{-1}; Anal. Calcd for $C_{16}H_{34}O_2Si$: C, 67.07; H, 11.96. Found: C, 66.89; H, 11.89; $[\alpha]^{25}_D$ +23.2, $[\alpha]^{25}_{577}$ +25.1, $[\alpha]^{25}_{546}$ +29.2, $[\alpha]^{25}_{435}$ +52.9, $[\alpha]^{25}_{405}$ +66.1, (*c* 1.25, $CHCl_3$).

Following the general procedure of Corey (Singh et al., supra, incorporated by reference herein) iodine (5.03 g, 19.8 mmol) was added in portions over 15 min to a 0°C solution of (S)-(Z)-5-triisopropylsiloxy-3-heptenol (5.17 g, 18.0 mmol), PPh_3 (5.19 g, 19.8 mmol), imidazole (1.35 g, 19.8 mmol) and Et_2O-MeCN (3:1, 135 mL) and then allowed to warm to 23°C. After 1 h the solution was partitioned between H_2O (150 mL) and Et_2O (150 mL). The

aqueous phase was extracted with Et_2O (2 x 150 mL). The combined organic extracts were then washed with Na_2SO_3 (150 mL) and H_2O (150 mL), dried ($MgSO_4$) and filtered. Purification of the crude product by flash chromatography (95:5 hexanes-Et_2O) afforded 6.67 g (94%) of iodide **75** as a colorless oil: ^1H NMR (500 MHz, $CDCl_3$) δ 5.49-5.53 (m, 1 H), 5.28-5.32 (m, 1 H), 4.41 (dd, J = 7.1, 5.9 Hz, 1 H), 3.10-3.14 (m, 2 H), 2.59-2.66 (m, 2 H), 1.58-1.62 (m, 1 H), 1.48-1.52 (m, 1 H), 1.05 (s, 21 H), 0.86 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) 136.2, 126.9, 70.0, 32.2, 31.6, 18.1, 12.3, 9.3, 4.4 ppm; IR (film) 3012, 2942, 1464, 1105, 883 cm^{-1}; Anal. Calcd for $C_{16}H_{33}OSiI$: C, 48.48; H, 8.39. Found: C, 48.63; H, 8.49; $[\alpha]^{25}_D$ +22.8, $[\alpha]^{25}_{577}$ +24.4, $[\alpha]^{25}_{546}$ +23.7, $[\alpha]^{25}_{435}$ +53.1, $[\alpha]^{25}_{405}$ +65.8, (*c* 1.2, $CHCl_3$).

Synthesis of (R)-Triethylsiloxy-N-methoxy-N-methyl-7-methyl-6-octenamide (Compound 76). To a 0°C solution of the known (Noyori, R. et. al. J. Am. Chem. Soc. 1987, 109, 5868) β-hydroxyester (10.0g, 53.5 mmol) in dry THF (200 mL) was added N, O-dimethylhydroxylamine hydrochloride (14 g, 64.2 mmol, 1.2 eq) followed by a 2 M solution of trimethylaluminum in toluene (60 mL, 2.3 eq) (added dropwise via cannula). The mixture was allowed to warm to room temperature and maintained at this temperature for 3 h. Then the mixture was (carefully) poored into a cold (0°C) 2 M solution of tartaric acid (500 mL). The resulting mixture was stirred for 5 h, after which the layers were separated and the aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Purification of the residue on silica gel yielded 10.2 g (88%) of Weinreb amide. The Weinreb amide (10.2 g, 47.5 mmol) was dissolved in CH_2Cl_2 (150 mL) and treated with Hünig's base (25 mL, 3eq). TESCl (8.6g, 9.7 mL, 1.2eq) was then added dropwise to the mixture. The progress of the reaction was monitored by TLC (hexanes, EtOAc, 3:1), and, upon completion, the mixture was diluted with water, the layers separated and the aqueous layer extracted with Et_2O (3 x 100 mL). The combined organic layers were washed with 0.5 N HCl (2 x 100 mL) and water (2 x 100 mL), dried ($MgSO_4$), and concentrated. The residue was purified on silica gel (hexane-EtOAc, 3:1), to give 12.9 g (82%) of **76** as a pale yellow oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.05 (m, 1H), 4.20-4.10 (m, 1H), 3.65 (s, 3H), 3.14 (s, 3H), 2.8-2.6 (dd, J= 17, 2 Hz, 1H), 2.3-2.4 (dd, J= 17, 3 Hz, 1H),

2.1-1.9 (m, 2H), 1.65 (s, 3H), 1.55 (s, 3H), 1.55-1.4 (m, 2H), 1.0-0.9 (m, 9H), 0.6-0.4 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) d 172.38, 131.50, 124.10, 69.10, 61.17, 39.61, 37.96, 31.90, 25.55, 23.81, 17.56, 6.80, 5.24.

Synthesis of (6R, 11Z, 13S)-2-Methyl-6-triethylsilyloxy-13-trisopropylsiloxypentadeca-2,11-dien-8-one (77). *t*-Buli (23.5 mL, 40.0 mmol, 1.7 M) was added to a -78°C solution of iodide **75** (6.67 g, 16.8 mmol) and Et_2O-hexanes (1:1, 100 mL). The solution was maintained at -78°C for 30 min, then a solution amide **76** (6.10 g, 18. 5 mmol) and Et_2O-hexanes (1:1, 40 mL) was added. The resulting solution was maintained at -78°C for 30 min then allowed to warm to 0°C and maintainedfor 2 h. The solution was then added to saturated aqueous NH_4Cl (150 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (2 x 150 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. Purification of the crude product by flash chromatography (98:2 hexanes-Et_2O) afforded 5.93 g (65%) of **77** as a clear oil. The product was ca. 95% pure and was used without further purification. A small sample was further purified by flash chromatography (98:2 hexanes-Et_2O) to obtain the following data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41-5.36 (m, 1 H), 5.29-5.24 (m, 1 H), 5.08 (tt, J = 7.1, 1.3 Hz, 1 H), 4.45 (app q, J = 6.7 Hz, 1 H), 4.18 (quintet, J = 6.0 Hz, 1 H), 2.60 (A of ABX, JAB =3D 15.3, JAX =3D 7.2 Hz, 1 H), 2.48-2.43 (m, 3 H), 2.30-2.24 (m, 2 H), 2.05-1.93 (m, 2 H), 1.68 (s, 3 H), 1.64-1.40 (m, 4 H), 1.59 (s, 3 H), 1.04 (s, 21 H), 0.94 (t, J = 7.9 Hz, 9 H), 0.85 (t, J = 7.5 Hz, 3 H), 0.58 (q, J = 7.9 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.9, 135.1, 131.8, 126.7, 123.8, 69.8, 68.7, 50.2, 44.1, 37.9, 31.6, 25.7, 23.8, 21.9, 18.1, 18.0, 17.6, 12.3, 9.3, 6.9, 4.9 ppm; IR (film) 2958, 2867, 1717, 1463, 1378, 1086, 1014 cm^{-1}; MS: HRMS (FAB) *m/z* 37.4141 (M-H), (537.4159 calcd for $C_{31}H_{61}O_3Si_2$); $[\alpha]^{25}_D$ +4.1, $[\alpha]^{25}_{577}$ +4.8, $[\alpha]^{25}_{546}$ +4.9, $[\alpha]^{25}_{435}$ +11.0, $[\alpha]^{25}_{405}$ +14.3 (*c* 1.6, $CHCl_3$).

Synthesis of (6R, 11Z, 13S)-8-(1',3'-dioxan-2'-yl)-6-hydroxy-2-methyl-13-trisopropylsiloxypentadeca-2,11-diene (79). A solution of ketone **77** (3.74 g, 6.94 mmol), orthoester **78** (4.10 g, 34.7 mmol), 1,3-propanediol (12.6 mL, 174 mmol), Amberlyst-15 resin (278 mg) and CH_3CN (70 mL) was maintained at rt for 7 h. The mixture was then filtered

through Celite and the filtrate was partitioned between Et_2O (150 mL) and H_2O (50 mL). The phases were separated, and the organic phase was washed with H_2O (250 mL), dried ($MgSO_4$), filtered and concentrated. Purification of the crude product by flash chromatography (85:15 hexanes-Et_2O) afforded 2.68 g (80%) of ketal **79** as clear oil: ^1H NMR (500 MHz, $CDCl_3$) δ 5.42-5.29 (m, 2 H), 5.14 (broad t, J = 7.1 Hz, 1 H), 4.45 (app q, J = 7.5 Hz, 1 H), 4.11-4.08 (m, 1 H), 4.02-3.85 (m, 4 H), 3.80 (s, 1 H), 2.16-1.96 (m, 6 H), 1.84-1.76 (m, 202 H), 1.68 (s, 3 H), 1.65-1.36 (m, 6 H), 1.61 (s, 3 H), 1.05 (s, 21 H), 0.86 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.6, 131.5, 127.5, 124.3, 101.1, 69.9, 67.0, 59.5, 59.5, 43.7, 37.5, 31.7, 31.3, 25.7, 25.2, 24.1, 22.3, 18.1, 18.0, 17.6, 12.4, 9.3 ppm; IR (film) 3532, 2960, 2866, 1464, 1381, 1246, 1109 cm^{-1}; MS: HRMS (FAB) *m/z* 505.3683 (M+Na), (505.3691 calcd for $C_{28}H_{54}O_4SiNa$). Anal. Calcd for $C_{28}H_{54}O_4Si$: C, 69.65; H, 11.27. Found: C, 69.40; H, 11.28; $[\alpha]^{25}_D$ +13.3, $[\alpha]^{25}_{577}$ +14.2, $[\alpha]^{25}_{546}$ +16.8, $[\alpha]^{25}_{435}$ +30.1, $[\alpha]^{25}_{405}$ +37.4 (*c* 1.6, $CHCl_3$).

Synthesis of (6S, 11Z, 13S)-6-amino-8-(1',3'-dioxan-2'-yl)-2-methyl-13-trisopropylsiloxypentadeca-2,11-diene (80). Triphenylphosphine (2.89 g, 11.0 mmol) and hydrazoic acid (5.82 mL, 12.1 mmol, 2.08 M in toluene) were added to a 0°C solution of alcohol **79** (2.65 g, 5.49 mmol) and THF (55 mL), then diethylazodicarboxylate (DEAD) (2.60 mL, 16.5 mmol) was added dropwise over a period of 15 min. The solution was maintained at 0°C for 1.5 h, then approximately half of the solvent was removed in vacuo. The resulting solution was diluted with hexanes (30 mL) and filtered through a plug of silica gel using 97:3 hexanes-Et_2O as the eluant. The filtrate was concentrated, and the crude product was purified by flash chromatography (97:3 hexanes-Et2O) affording 2.45 g (88%) of the azide as a clear oil: ^1H NMR (500 MHz, $CDCl_3$) δ 5.41-5.29 (m, 2 H), 5.10 (broad t, J = 7.1 Hz, 1 H), 4.47 (app q, J = 7.4 Hz, 1 H), 3.96-3.86 (m, 4 H), 3.71-3.66 (m, 1 H), 2.12-2.07 (m, 3 H), 2.00-1.72 (m, 6 H), 1.70 (s, 3 H), 1.64 (s, 3 H), 1.63-1.42 (m, 5 H), 1.05 (s, 21 H), 0.87 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.6, 132.4, 127.7, 123.3, 99.1, 69.9, 59.6, 59.6, 58.3, 42.2, 36.1, 32.2, 31.7, 25.7, 25.1, 24.7, 22.2, 18.1, 18.1, 17.6, 12.4, 9.4 ppm; IR (film) 2961, 2866, 2101, 1463, 1381, 1246, 1145, 1110 cm^{-1}; MS: HRMS (FAB) (M - H) *m/z* 506.3776, (506.3781 calcd for $C_{28}H_{52}N_3O_3Si$). Anal. Calcd for $C_{28}H_{53}N_3O_3Si$: C,

66.22; H, 10.52. Found: C, 66.27; H, 10.50. $[\alpha]^{25}_{D}$ +9.5, $[\alpha]^{25}_{577}$ +10.3, $[\alpha]^{25}_{546}$ +12.1, $[\alpha]^{25}_{435}$ +24.1, $[\alpha]^{25}_{405}$ +31.2 (*c* 1.6, $CHCl_3$).

A solution of the above azide (2.45 g, 4.82 mmol) and Et_2O (18 mL) was added to a 0°C solution of $LiAlH_4$ (12.1 mL, 12.1 mmol, 1.0 M in Et_2O) and Et_2O (18 mL). The ice bath was removed, and the solution was allowed to warm to rt. After 1 h the reaction was quenced by sequential addition of H_2O (600 μL), NaOH (600 μL, 3 N) and H_2O (1.8 mL). The resulting mixture was stirred for 1 h, then $MgSO_4$ was added. The mixture was filtered through celite and concentrated to afford a brown oil. Purification of the crude product by flash chromatography (10:1:0.1 $CHCl_3$-MeOH-conc. NH_4OH) afforded 2.05 g (88%) of amine **80** as a light yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39-5.29 (m, 2 H), 5.11 (br t, *J* = 7.1 Hz, 1 H), 4.46 (app q, *J* = 7.4 Hz, 1 H), 3.95-3.84 (m, 4 H), 3.15-3.11 (m, 1 H), 2.10-1.96 (m, 4 H), 1.83-1.69 (m, 4 H), 1.68 (s, 3 H), 1.63-1.31 (m, 6 H), 1.61 (s, 3 H), 1.05 (s, 21 H), 0.86 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.3, 131.4, 127.9, 124.1, 100.4, 69.8, 59.4, 59.2, 46.7, 43.1, 38.8, 32.7, 31.6, 25.6, 25.3, 24.6, 22.1, 18.0, 18.0, 17.6, 12.3, 9.3 ppm; IR (film) 3387, 3310, 2942, 2866, 1464, 1381, 1366, 1246, 1109 cm^{-1}; MS: HRMS (FAB) (M + H^+) *m/z* 482.4011, (482.4029 calcd for $C_{28}H_{56}NO_3Si$). Anal. Calcd for $C_{28}H_{55}NO_3Si$: C, 69.80; H, 11.51. Found: C, 69.85; H, 11.56; $[\alpha]^{25}_{D}$ +21.2, $[\alpha]^{25}_{577}$ +22.7, $[\alpha]^{25}_{546}$ +26.1, $[\alpha]^{25}_{435}$ +47.2, $[\alpha]^{25}_{405}$ +58.1 (*c* 1.6, $CHCl_3$).

Synthesis of (6S, 11Z, 13S)-8-(1',3'-Dioxan-2'-yl)-2-methyl-13-trisopropylsiloxy-6-uriedopentadeca-2,11-diene (81). Trimethylsilyl isocyanate (0.55 mL, 4.1 mmol) was added to a rt solution of **80** (1.61 g, 3.35 mmol), CH_2Cl_2 (6.8 mL) and i-PrOH (0.31 mL). After 15 h, i-PrOH (3 mL) was added and the solution was maintained for 1 h, then concentrated. The resulting oil was purified on silica gel (100% EtOAc) to provide 1.57 g (89%) of **81** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl^3$) δ 5.24 – 5.36 (m, 2H), 5.03-5.15 (m, 4H), 4.41 (dd, *J* = 13.2, 7.1 Hz, 1H), 3.80-3.91 (m, 4H), 3.64 (m, 1H), 1.71-2.03 (m, 8H), 1.63 (s, 3H), 1.55 (s, 3H), 1.36-1.63 (m, 6H), 1.00 (s, 21H), 0.82 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 159.3, 134.4, 131.8, 127.5, 123.7, 99.9, 69.7, 59.4, 59.2, 46.7, 36.9, 31.5, 31.1, 25.6, 25.0, 24.5, 22.1, 17.9, 17.8, 17.5, 12.2, 9.2 ppm; IR(film) 3354, 2960, 1660,

1600, 1556, 1463, 1381, 1109 cm^{-1}; $[\alpha]^{25}_{D}$ +7.0,$[\alpha]^{25}_{577}$ +12.0, $[\alpha]^{25}_{546}$ +17.3, $[\alpha]^{25}_{435}$ +20.7, $[\alpha]^{25}_{405}$ +25.4, (*c* 1.05, $CHCl_3$). Anal. Calcd for $C_{29}H_{56}N_2O_4Si$: C, 66.36; H, 10.75; N, 5.34. Found: C, 66.31; H, 10.70; N 5.41.

Synthesis of (4aR,7S)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-1,2,4a,5,6,7-hexahydro-3-[(4S)-tert-butyldimethylsiloxypentyl)]-7-[(7S, Z)-2-(1',3'-dioxan-2'-yl)-7-triisopropylsiloxynon-5-enyl)]-1-oxo-pyrrolo[1,=2-c] pyrimidine (83). Osmium tetroxide (0.75 mL, 0.1 M in t-BuOH) was added to a solution of **81** (524 mg, 1.00 mmol), NMO (406 mg, 3.46 mmol), and 10:1 THF-H_2O (25 mL). After 1.5 h, florisil (3 g), $NaHSO_3$ (3 g), and EtOAc (50 mL) were added and the reaction mixture was stirred vigouously. After 30 min, the reaction mixture was filtered, and the filtrate concentrated to provide a colorless oil which was used without further purification.

A solution of this crude diol, $Pb(OAc)_4$ (532 mg, 1.20 mmol), and toluene (60 mL) was maintained at room temperature. The reaction mixture was filtered through a plug of Celite,® morpholinium acetate (300 mg, 2.0 mmol) was added, and the solution concentrated to provide the crude aminal 82 as a slightly yellow oil.

A solution of this crude aminal, **47** (1.95 g, 3.36 mmol) and 2,2,2-trifluoroethanol (1.0 mL) was maintained at 60°C for 2 d. The reaction was quenched by adding Et_2O (20 mL) and 50% aqueous NH_4Cl (5 mL). The layers were separated, the organic layer was dried ($MgSO_4$), concentrated, and the resulting oil purified on silica gel (10:1 hexanes-EtOAc; 7:1 hexanes-EtOAc; 3:1 hexanes-EtOAc) to provide 1.5 g of 24 and 638 mg (61%) of a ~6.5:1 mixture of **83** and **84**, which was used without separation. For characterization purposes, a 50 mg sample of this mixure was purified by HPLC (7:1 hexanes-EtOAc; Altima 5 _ silica). 60: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.72 (s, 1H), 5.87-5.95 (m, 1H), 5.21-5.37 (m, 4H), 4.56 (d, *J* = 5.7 Hz, 2H), 4.51 (dd, *J* = 12.7, 7.1 Hz, 1H), 4.22 (dd, *J* = 11, 4.6 Hz, 1H), 4.06-4.13 (m, 3H), 3.97-3.98 (m, 1H), 3.76-3.88 (m, 4H), 2.47-2.58 (m, 3H), 2.39 (d, *J* = 13.6 Hz, 1H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.26-2.32 (m, 1H), 2.15 (dd, *J* = 13.0, 6.0 Hz, 1H), 1.99-2.03 (m,

1H), 1.50-1.90 (m, 13H), 1.41-1.48 (m, 3H), 1.11-1.40 (m, 23H), 1.10 (d, J= 6. 1 Hz, 3H), 0.91-1.07 (m, 21H), 0.82-0.91 (m, 12H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 173.5, 166.0, 151.9, 151.2, 134.3, 132.2, 128.2, 118.0, 102.1, 99.2, 69.9, 68.3, 64.9, 64.2, 59.3, 57.7, 52.7, 39.0, 37.4, 34.5, 34.2, 31.8, 31.3, 30.4, 29.6, 29.57, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 26.1, 25.9, 25.3, 24.9, 24.4, 23.6, 21.8, 18.1, 12.3, 9.3, -4.4, -4.7 ppm; IR (film) 3211, 3095, 2927, 2856, 1741, 1682, 1627, 1463, 1435, 1107 cm^{-1}; $[\alpha]^{25}_{D}$ -4.5, $[\alpha]^{25}_{577}$ -4.9, $[\alpha]^{25}_{546}$ -5.7, $[\alpha]^{25}_{435}$ -15.5, $[\alpha]^{25}_{405}$ -22.7, (*c* 0.75, $CHCl_3$). Anal. Calcd for $C_{59}H_{108}N_{209}Si_2$: C, 67.77; H, 10.41; N, 2.68. Found: C, 67.68; H, 10.27; N 2.65.

(3R,4R,4aR,6'R,7S)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-1,2,4a,5,6, 7-hexahydro-1-oxo-7-[(7S, 5Z)-7-hydroxy-2-oxo-5-nonenyl]-pyrrolo[1,2-c]pyrimidine-3-spiro-6'-(2'-methyl)-3',4',5',6'-tetrahydro-2H-pyran (85). A solution of **83** (1.30 g, 1.24 mmol), TBAF (6.22 mL, 1.0 M solution in Et_2O), and DMF (31 mL) was maintained at rt for 5 h. The solution was diluted with Et_2O (150 mL) and washed with H_2O (50 mL) and brine (250 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate concentrated. The resulting residue was used without further purification.

A solution of this crude diol, $TsOH{\cdot}H_2O$ (236 mg, 1.24 mmol), and $CHCl_3$ (180 mL) was maintained at 60°C for 15 min. The reaction was quenched by adding saturated aqueous $NaHCO_3$ (20 mL). The layers were separated and the organic layer was washed with brine (20 mL), then the organic layer was dried ($MgSO_4$), concentrated, and the resulting oil purified on silica gel (1:3 hexanes-EtOAc; 100% EtOAc) to provide 630 mg (71%) of a ~6.5:1 mixture isomers. 62: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87-5.95 (m, 1H), 5.56 (s, 1H), 5.34-5.43 (m, 2H), 5.31 (dd, J = 17.2, 1.5 Hz, 1H), 5.22 (dd, J = 10.6, 1.3 Hz, 1H), 4.57 (dd, J = 4.3, 1.3 Hz, 2H), 4.38 (dd, J = 14.5, 6.8 Hz, 1H), 4.29-4.31 (m, 1H), 4.08-4.18 (m, 2H), 4.02 (dt, J =11.1, 4.8 Hz, 1H), 3.77-3.80 (m, 1H), 3.37 (d, J = 16.8 Hz, 1H), 2.52-2.60 (m, 2H), 2.43-2.50 (m, 1H), 2.32 (t, J = 7.5 Hz, 2H), 2.22-2.27 (m, 2H), 2.04-2.20 (m, 4H), 1.69-1.76 (m, 4H), 1.56-1.65 (m, 7H), 1.42-1.48 (m, 3H), 1.24-1.28 (m, 21H), 1.06-1.09 (m, 1H), 1.05 (d, J = 6.0 Hz, 3H), 0.89 (t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 209.0, 173.5, 168.9, 153.0, 134.1, 132.3, 129.8, 118.1, 82.2, 68.4, 66.2, 65.1, 64.9, 55.0, 54.0, 53.2,

46.2, 42.7, 34.3, 32.2, 32.1, 30.3, 30.0, 29.6, 29.57, 29.5, 29.4, 29.3, 29.2, 29.1, 28.7, 26.0, 24.9, 22.0, 21.7, 18.8 ppm; IR (film) 3450, 3231, 3081, 2927, 2855, 1732, 1715, 1659, 1651, 1470, 1373, 1262, 1013 cm^{-1}; $[\alpha]^{25}_{D}$ +42.2, $[\alpha]^{25}_{577}$ +42.7, $[\alpha]^{25}_{546}$ +49.8, $[\alpha]^{25}_{435}$ +91.0, $[\alpha]^{25}_{405}$ +114, (*c* 0.60, $CHCl_3$). Anal. Calcd for $C_{41}H_{68}N_2O_8$: C, 68.68; H, 9.56; N, 3.91. Found: C, 68.71; H, 9.51; N 3.84.

Synthesis of (3R,4R,4aR,6'R,7S)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-1,2,4a,5,6,7-hexahydro-1-oxo-7-[(7S, 5Z)-7-chloroacetoxy-2-oxo-5-nonenyl]-pyrrolo[1,2-c]pyrimidine-3-spiro-6'-(2'-methyl)-3',4',5',6'-tetrahydro-2H-pyran (86). Chloroacetyl chloride (0.34 mL, 0.46 mmol) was added dropwise to a 0°C solution of **85** (0.63 g, 0.88 mmol), pyridine (1.42 mL, 17.6 mmol), and CH_2Cl_2 (50 mL). The solution was immediately allowed to warm to rt. After 1 h, the solution was quenched by adding Et_2O (200 mL) and washed with 1N NaOH (25 mL), $CuSO_4$ (225 mL), and brine (25 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate concentrated. The resulting residue was purified on silica gel (2:1 hexanes-EtOAc; 1:1 hexanes-EtOAc; 1:2 hexanes-EtOAc) to yield 600 mg (86%) of the desired cis isomer **86** as a colorless oil, and ~85 mg (~12%) of an undesired trans isomer which was derived from **84**. **86**: ^1H NMR (500 MHz, $CDCl_3$) δ 6.34 (s, 1H), 5.87-5.94 (m, 1H), 5.48-5.56 (m, 2H), 5.27-5.32 (m, 2H), 5.22 (d, *J* = 10.4 Hz, 1H), 4.56 (d, *J* = 5.7 Hz, 2H), 4.31-4.33 (m, 1H), 4.09-4.19 (m, 2H), 4.03 (s, 2H), 4.00-4.06 (m, 1H), 3.77-3.81 (m, 1H), 3.34 (d, *J* = 16.6 Hz, 1H), 2.40-2.48 (m, 3H), 2.25-2.38 (m, 5H), 2.05-2.17 (m, 3H), 1.69-1.74 (m, 4H), 1.55-1.62 (m, 7H), 1.42-1.50 (m, 1H), 1.24-1.31 (m, 22H), 1.06-1.15 (m, 1H), 1.05 (d, *J* = 6.0 Hz, 3H), 0.89 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 207. 9, 173.4, 168.8, 166.6, 153.4, 133.0, 132.3, 127.8, 118.0, 82.1, 73.7, 66.1, 64.9, 64.8, 54.9, 53.9, 53,1, 46.3, 42.3, 41.1, 34.2, 32.2, 32.0, 29.5, 29.49, 29,4, 29.3, 29.2, 29.1, 29.07, 29.0, 28.6, 27.4, 25.9, 21.8, 21.6, 18.5, 9.3 ppm; IR (film) 3296, 2928, 2855, 1732, 1652, 1466, 1303, 1174, 1013 cm^{-1}; $[\alpha]^{25}_{D}$ +42.7, $[\alpha]^{25}_{577}$ +47.0, $[\alpha]^{25}_{546}$ +52.6, $[\alpha]^{25}_{435}$ +96.1, $[\alpha]^{25}_{405}$ +120, (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{43}H_{69}N_2O_9Cl$: C, 65.09; H, 8.77; N, 3.53. Found: C, 65.16; H, 8.79; N 3.57.

Synthesis of Pentacyles 87 and 88. A solution of **86** (327 mg, 0.412 mmol), MeOTf (1.29 mL, 8.21 mmol), 2,6-di-t-butylpyridine (0.46 mL, 2.1 mmol), and CH_2Cl_2 (20 mL) was maintained at room temperature for 8 h. The solution was then poured into Et_2O (100 mL) and washed with 1 N NaOH (2 x 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate concentrated. The resulting residue was used without further purification.

Ammonia was bubbled through a room temperature solution of the above crude pseudourea, N_4HCl (50 mg, 0.93 mmol), and allyl alcohol (5 mL) for 20 min (saturated solution). The reaction vessel was sealed and heated to 60°C for 28 h. The reaction mixture was then cooled rt, concentrated, and the resulting oil purified by silica gel MPLC (100:0.6 $CHCl_3$ -*i*-PrOH) to provide a 147 mg of **87** and 98 mg of **88**. **87** was twice recycled through the above guanylation reaction conditions to yield an additional 60 mg of **88** (52% combined yield). **87**: ^1H NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.56 (s, 1H), 5.88-5.95 (m, 1H), 5.64-5.67 (m, 1H), 5.48 (d, J = 10.9 Hz, 1H), 5.33 (dd, J = 17.2, 1.5 Hz, 1H), 5.25 (dd, J = 10.4, 1.2 Hz, 1H), 4.57 (d, J = 5.7 Hz, 2H), 4.48 (d, J = 10.3 Hz, 1H), 4.32-4.38 (m, 1H), 4.10-4.24 (m, 3H), 3.78-3.81 (m, 1H), 2.56-2.61 (m, 2H), 2.45 (d, J = 11.6 Hz, 1H), 2.32 (t, J = 7.6 Hz, 2H), 2.26-2.36 (m, 3H), 2.15-2.18 (m, 2H), 2.00 (dt, J = 13.8, 4.7 Hz, 1H), 1.87 (dd, J = 14.6, 5.4 Hz, 1H), 1.61-1.78 (m, 10H), 1.53-1.58 (m, 1H), 1.42-1.49 (m, 1H), 1.23-1.35 (m, 22H), 1.05-1.15 (m, 1H), 1.05 (d, J = 6.1 Hz, 3H), 0.81 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 173. 5, 167.6, 147.3, 133.4, 132.3, 129.7, 118.0, 83.9, 81.7, 70.9, 67.6, 65.7, 64.9, 53.4, 53.3, 36.8, 36.2, 34.2, 31.8, 30.6, 29.7, 29.6, 29.5, 29.46, 29.4, 29.2, 29.1, 29.08, 29.0, 28.5, 25.9, 24.9, 23.6, 21.3, 17.9, 10.1 ppm; IR (film) 3268, 3147, 3020, 2927, 2855, 1732, 1660, 1608, 1465, 1283, 1164, 1029 cm^{-1}; $[\alpha]^{25}_D$ +12.2, $[\alpha]^{25}_{577}$ +13.1, $[\alpha]^{25}_{546}$ +14.1, (c 2.00, $CHCl_3$). HRMS (FAB) *m/z* 698.5108 cald for $C_{41}H_{68}N_3O_6$, found 698.5096 [M]. **88**: ^1H NMR (500 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.43 (s, 1H), 5.88-5.95 (m, 1H), 5.64-5.67 (m, 1H), 5.48 (d, J = 10.9 Hz, 1H), 5.31 (dd, J = 17.2, 1.5 Hz, 1H), 5.22 (dd, J = 10.4, 1.2 Hz, 1H), 4.57 (dd, J= 5.7, 1.2 Hz, 2H), 4.48 (d, J= 9.7 Hz, 1H), 4.29-4.33 (m, 1H), 4.08 (t, J = 6.8 Hz, 2H), 3.99-4.05 (m, 1H), 3.84-3.87 (m, 1H), 2.93 (d, J = 4.8 Hz, 1H), 2.55-2.63 (m, 2H), 2.32 (t, J = 7.6 Hz, 2H), 2.26-2.36 (m, 2H), 2.13-2.24 (m, 3H), 1.98 (dd, J = 14.7, 5.3 Hz, 1H),

1.78-1.84 (m, 1H), 1.51-1.76 (m, 10H), 1.38-1.48 (m, 2H), 1.21-1.30 (m, 22H), 1.07-1.20 (m, 1H), 1.05 (d, J = 6.1 Hz, 3H), 0.81 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 173.5, 168.3, 148.4, 133.5, 132.3, 129.7, 118.0, 84.0, 80.8, 70.9, 67.2, 65.5, 64.9, 54.1, 52.2, 49.9, 36.7, 36.1, 34.4, 31.8, 31.7, 30.5, 29.6, 29.56, 29.5, 29.45, 29.4, 29.2, 29.1,29.06, 28.4, 26.7, 25.8, 24.9, 23.6, 21.5, 17.7, 10.0 ppm; IR (film) 3263, 3154, 3025, 2928, 2854, 1732, 1652, 1614, 1516, 1464, 1298 cm^{-1}; $[\alpha]^{25}_{D}$ -9.6, $[\alpha]^{25}_{577}$ -10.5, $[\alpha]^{25}_{546}$ -9.5, $[\alpha]^{25}_{435}$ -16.5, $[\alpha]^{25}_{405}$ -17.2, (*c* 0.75, $CHCl_3$). HRMS (FAB) *m/z* 698.5108 cal'd for $C_{41}H_{68}N_3O_6$, Found 698.5106 [M].

Synthesis of Carboxylic Acid 89: A solution of **88** (27 mg, 37 μmol), $Pd(PPh_3)_4$ (21 mg, 18 μmol), morpholine (13 μL, 0.15 mmol), and MeCN (1.0 mL) was maintained at rt for 5 h. The solution was diluted with Et_2O (30 mL), and washed with 0.1 N HCl (25 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate concentrated. The resulting residue was purified on silica gel (100:1 $CHCl_3$-MeOH; 33:1 $CHCl_3$ -MeOH) to yield 24 mg (94%) of the desired product **89** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.63-5.66 (m, 1H), 5.46-5.49 (m, 1H), 4.48 (app d, J = 10.2 Hz, 1H), 4.27-4.31 (m, 1H), 4.04-4.12 (m, 2H), 3.96-4.03 (m, 1H), 3.85-3.88 (m, 1H), 2.92 (d, J = 4.9 Hz, 1H), 2.62 (t, J = 13.8 Hz, 1H), 2.55 (dd, J = 12.7, 4.7 Hz, 1H), 2.12-2.32 (m, 7H), 1.86 (dd, J = 14.8, 5.3 Hz, 1H), 1.77-1.81 (m, 1H), 1.60-1.73 (m, 9H), 1.51-1.59 (m, 1H), 1.37-1.45 (m, 2H), 1.20-1.30 (m, 22H), 1.16-1.20 (m, 1H), 1.04 (d, J = 6.1 Hz, 3H), 0.80 (t, J = 7.2 Hz, 3H), the NH and OH signals are not observable; ^{13}C NMR (125 MHz, $CDCl_3$) 179.1, 168.4, 148.7, 133.6, 129.8, 83.9, 80.8, 70.8, 67.0, 65.4, 54.0, 52.0, 50.0, 36.7, 36.0, 31.9, 31.8, 30.5, 29.4, 29.3, 29.29, 29.26, 29.1, 29.0, 28.4, 26.7, 25.8, 25.5, 23.7, 21.5, 17.8, 10.0 ppm; IR (film) 3261, 3138, 2919, 2849, 1728, 1658, 1606, 1465, 1284, 1154, 1031 cm^{-1}; $[\alpha]^{25}_{D}$ -17.7, $[\alpha]^{25}_{577}$ -17.0, $[\alpha]^{25}_{546}$ -18.7, $[\alpha]^{25}_{435}$ -28.5, (*c* 1.10, $CHCl_3$). HRMS (FAB) *m/z* 658.4795 cal'd for $C_{38}H_{64}N_3O_6$, found 658.4791 [M].

Synthesis of 41,45-bis-t-Butoxycarbony Crambescidin 800 (91). Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (22 mg, 50 μmol) was added to a rt solution of carboxylic acid **89** (23 mg, 33 μmol), amine **90** (18 mg,

50 μmol), Et_2N (0.15 mL, 1.1 mmol), and CH_2Cl_2 (5 mL). After 4 h, the reaction was diluted with Et_2O (20 mL), and washed with saturated aqueous NH_4Cl (5 mL) and brine (5mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated. The resulting residue was purified on silica gel (50:1 $CHCl_3$ -MeOH) to yield 28 mg (82%) of the desired product **91** as a colorless oil: ^{1}H NMR (500 MHz, d4-MeOD) δ 5.70-5.73 (m, 1H), 5.47-5.52 (m, 1H), 4.40 (br d, J = 10.3 Hz, 1H), 4.33-4.37 (m, 1H), 4.10-4.16 (m, 2H), 4.02-4.09 (m, 1H), 3.75-3.85 (m, 2H), 3.34-3.59 (m, 2H), 3.23-3.29 (m, 2H), 3.12-3.20 (m, 2H), 3.07 (d, J = 4.8 Hz, 1H), 2.94-3.06 (m, 2H), 2.64 (dd, J = 13.0, 4.7 Hz, 1H), 2.26-2.46 (m, 6H), 2.10-2.20 (m, 1H), 2.00 (dd, J = 13.9, 5.8 Hz, 1H), 1.79-1.85 (m, 3H), 1.50-1.77 (m, 11H), 1.36-1.47 (m, 20H), 1.22-1.35 (m, 25H), 1.09 (d, J = 6.1 Hz, 3H), 0.85 (t, J = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, d4-MeOD) 176.6/176.2, 170.2, 158.5, 150.2, 134.3, 131.3, 85.1, 82.2, 80.0, 79.96, 72.3, 69.1, 68.4, 68.37, 66.5, 55.6, 55.0, 54.2, 53.5, 50.7, 45.1, 38.9, 38.7, 38.3, 38.1, 37.9, 36.2, 34.3, 34.1, 33.0, 32.6, 31.5, 30.8, 30.7, 30.6, 30.5, 30.3, 30.2, 29.6, 28.8, 28.7, 27.6, 27.0, 26.7, 26.6, 24.4, 21.8, 19.5, 10.8 ppm; IR (film) 3356, 2934, 2858, 1732, 1706, 1657, 1613, 1509, 1459, 1251, 1170 cm^{-1}; $[\alpha]^{25}_D$ -3.0, $[\alpha]^{25}_{577}$ -2.2, $[\alpha]^{25}_{546}$ -2.8, $[\alpha]^{25}_{435}$ -3.5, $[\alpha]^{25}_{405}$ -3.6, (*c* 0.75, $CHCl_3$). HRMS (FAB) m/z 1001.7 cald for $C_{55}H_{97}N_6O_{10}$, found 1001.7 [M].

Synthesis of Crambescidin 800 Trihydrochloride (2). A solution of **91** (13 mg, 13 μmol) and 1.3 mL of a 3.0 M solution of HCl in EtOAc was maintained at rt for 20 mins and then concentrated. Purification of the residue by reverse phase HPLC (4:1 MeOH-0.1 M NaCl, Altima C18, 5 column) gave ~11.8 mg (75%) of crambescidin 800 as its trihydrochloride salt (a light yellow oil). Data for this sample were consistent with data published for natural 2. Data for synthetic **2**: ^{1}H NMR (500 MHz, d4-MeOD) δ 5.71 (m, 1H), 5.50 (app d, J = 10.9 Hz, 1H), 4.41 (m, 1H), 4.33 (m, 1H), 4.13 (m, 1H), 4.05 (m, 1H), 3.96 (m, 1H), 3.85 (m, 1H), 3.65 (m, 2H), 3.55 (m, 1H), 3.44 (m, 2H), 3.11 (m, 2H), 3.07 (d, J = 4.8 Hz, 1H), 2.97 (m, 0.5H), 2.88 (m, 1.5H), 2.64 (dd, J = 12.8, 4.7 Hz, 1H), 2.23-2.51 (m, 7H), 2.17 (m, 1H), 1.50-2.10 (m, 15H), 1.42 (t, J = 12.2 Hz, 1H), 1.20-1.40 (m, 25H), 1.09 (d, J = 6. 1 Hz, 3H), 0.85 (t, J = 7.2 Hz, 3H); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.74 (s, 1H), 9.50 (s, 1H), 8.00 (br s, 6H), 5.67 (app s, 1H), 5.47 (app d, J = 10.4 Hz, 1H), 4.49 (m, 1H), 4.28 (m, 1H), 4.07 (m,

2H), 3.97 (m, 2H), 3.45-3.66 (m, 3H), 3.29 (m, 2H), 3.11 (m, 2H), 2.95 (m, 2H), 2.55 (m, 1H), 2.10-2.50 (m, 7H), 2.05 (m, 1H), 1.95 (m, 1H), 1.50-1.70 (m, 15H), 1.40-1.50 (m, 2H), 1.20-1.40 (m, 25H), 1.05 (d, J = 5.4 Hz, 3H), 0.83 (t, J = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, d4-MeOD) 177.5, 170.2, 150.2, 134.3, 131.3, 85.1, 82.1, 72.3, 69.4, 68.4, 66.5, 55.6, 54.8, 54.1, 50.7, 43.8, 38.5, 38.3, 38.2, 37.9, 33.0, 32.9, 32.6, 31.5, 30.8, 30.7, 30.67, 30.6, 30.3, 30.26, 29.6, 27.6, 27.0, 26.6, 26.55, 24.4, 21.8, 19.4, 10.8 ppm; ^{13}C NMR (125 MHz, $CDCl_3$) 175.5/175.0, 168.3, 148.7, 133.6, 129.8, 83.5, 80.6, 70.9, 67.1, 65.4, 54.4, 53.9, 51.8, 49.5, 44.0, 37.9, 37.6, 37.0, 36.9, 33.5, 33.2, 32.0, 31.8, 30.8, 30.6, 29.7, 29.6, 29.59, 29.55, 29.5, 29.1, 29.0, 28.4, 26.9, 25.8, 25.5, 25.4, 23.4, 21.4, 18.3, 10.1 ppm; IR (film) 3382, 3231, 2923, 2852, 1732, 1659, 1614, 1469, 1167, 1086, 1015 cm^{-1}; $[\alpha]^{25}_{D}$ -4.4, $[\alpha]^{25}_{577}$ -5.0, $[\alpha]^{25}_{546}$ -4.0, $[\alpha]^{25}_{435}$ -6.3, $[\alpha]^{25}_{405}$ -6.2, (c 0.70, $CHCl_3$). HRMS (FAB) m/z 801.6217 cald for $C_{45}H_{81}N_6O_6$, found 801.6222 [M].

Synthesis of Peracetylcrambescidin 800 (92). A solution of crambescidin 800 (2) (5.0 mg, 5.5 μg), Ac_2O (0.5 mL), and pyridine (1 mL) was maintained at rt for 23 then concentrated in vacuo (0.9 mm Hg, 23°C), diluted with $CHCl_3$ (20 mL) and washed with 0.1 M HCl (5 mL), and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated. The resulting residue was purified on silica gel (20:1 $CHCl_3$-MeOH; 10:1 $CHCl_3$-MeOH) to yield 2 mg (35%) of peracetylcrambescidin 800 (**92**) as a white wax. Data for this sample were consistent with data published for naturally derived peracetylcrambescidin 800. Data for synthetic **92**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88 (s, 1H), 9.64 (s, 1H), 6.75 (br s, 0.7H), 6.38 (br s, 0.7H), 6.18 (br s, 0.7H), 5.67 (app t, J = 10.5 Hz, 1H), 5.49 (app d, J = 11.0 Hz, 1H), 5.10 (m, 1H), 4.51 (m, 1H), 4.28 (app dt, J = 9.8, 4.9 Hz, 1H), 4.12 (m, 1H), 4.06 (m, 1H), 4.04 (m, 2H), 3.46-3.64 (m, 2H), 3.20-3.39 (m, 4H), 2.99-3.16 (m, 2H), 2.94 (d, J = 4.6 Hz, 1H), 2.55 (dd, J = 12.6, 4.6 Hz, 1H), 2.49 (m, 1H), 2.17-2.38 (m, 7H), 2.05 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.92-196 (m, 1H), 1.52-1.82 (m, 14H), 1.43 (t, J = 12.2 Hz, 1H), 1.20-1.40 (m, 25H), 1.05 (d, J = 6.1 Hz, 3H), 0.83 (t, J = 7.2 Hz, 3H); ^{1}H NMR (500 MHz, d4-MeOH) δ 5.72 (app t, J = 10.9 Hz, 1H), 5.51 (app d, J = 11.0 Hz, 1H), 5.15 (m, 1H), 4.32-4.39 (m, 2H), 4.13 (dt, J = 6.6, 1.8 Hz, 2H), 4.07 (m, 1H), 3.83 (m, 1H), 3.39-3.63 (m, 4H), 3.14-3.25 (m, 4H), 3.08 (d, J = 4.9 Hz, 1H), 2.64 (dd, J =

13.0, 4.8 Hz, 1H), 2.29-2.47 (m, 7H), 2.17 (m, 1H), 2.02 (s, 3H), 2.01 (m, 1H), 1.94 (s, 1.5H), 1.93 (s, 1.5H), 1.92 (s, 1.5H), 1.91 (s, 1.5H), 1.53-1.86 (m, 14H), 1.42 (t, J = 12.6 Hz, 1H), 1.20-1.40 (m, 25H), 1.09 (d, J = 6.2 Hz, 3H), 0.85 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 174.5, 171.0, 170.8, 170.5, 170.4, 168.3, 148.8, 133.7, 129.8, 83.6, 80.7, 71.0, 70.6, 67.3, 65.4, 53.9, 51.8, 50.5, 49.7, 42.6, 37.0, 36.96, 35.9, 35.3, 33.2, 32.4, 32.0, 30.6, 29.7, 29.6, 29.5, 29.4, 29.1, 28.5, 27.1, 26.8, 25.8, 25.5, 23.5, 21.4, 18.4, 10.1 ppm; IR (film) 3457, 3240, 2923, 1739, 1732, 1660, 1643, 1614, 1463, 1372, 1238, 1015 cm^{-1}; $[\alpha]^{25}_D$ -37 (*c* 0.2, $CHCl_3$). HRMS (FAB) *m/z* 927.6534 cald for $C_{51}H_{87}N_6O_9$, found 927.6547 [M].

These results demonstrate the first total synthesis of Crambescidin 800 (compound **2**) in a convergent fashion with the longest linear sequence from commercially available starting material in 25 steps and in a 3.0% overall yield. These experiments confirm the stereochemical assignment of Crambescidin 800 (compound **2**) and rigorously establish that the absolute configuration of it hydroxysperimidine side chain is S.

EXAMPLE IV

Initial Method for enantioselective total synthesis of 13,14,15-Isocrambescidin 800

Synthesis Plan. Molecular mechanics models of the methyl esters of the 13,14,15-Isocrambescidin 800 and Ptilomycalin A/Crambescidin are shown in Figure 20. These models clearly illustrate the structural differences and similarities between the two structures. For instance, the C10 and C13 angular hydrogens are *trans* in the Isocrambescidin core and *cis* in the Ptilomycalin A/Crambescidin core, but the relationship between the substituents at C13, C14 and C15 is the same in both structures. Also, the C–O bonds in both structures are axial. Thus, as in the Ptilomycalin A synthesis, we surmised that the C8 and C15 spirocenters could be constructed with the desired stereochemistry if the required *trans* stereochemistry of the central triazacenaphthalene ring system was in place. This strategy would require setting the *trans* relationship of the C10 and C13 angular hydrogens and relating this chirality to the C3 and C19 stereocenters of the oxopene and hydropyran rings.

The initial synthetic challenge was constructing the triazacenaphthalene ring system with the desired *trans* stereochemistry. In the (–)-Ptilomycalin A synthesis the required *cis* stereochemistry was established via a "tethered Biginelli" condensation of a ureido aldehyde and a β-ketoester. (See Overman, L. E.; Rabinowitz, M. H. *J. Org. Chem.* **1993**, *58*, 3235–3237; Overman, L. E.; Rabinowitz, M. H.; Renhowe P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2657–2658 and Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937–6963).

Further studies in our laboratories revealed that a Biginelli condensation between a tethered guanyl aldehyde and a β-ketoester afforded 1-iminohexahydropyrrolo[1,2-*c*]pyrimidine intermediates with the *trans* relationship about the pyrrolidine ring (Figure 30) (McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 1520–1528) thus providing a strategy for constructing the Isocrambescidin core.

A retrosynthetic analysis of the Isocrambescidin core is shown in Figure 21. Disconnection of the C8 and C15 aminals of **94** leads to a 1-iminohexahydropyrrolo[1,2-*c*]pyrimidine intermediate such as **95**. With the guanidine unit in place, we envisaged formation of the final three rings of the pentacyclic core directly from an intermediate such as **95**. We further envisaged **95** being formed via a Biginelli condensation of guanyl aldehyde **96** and a β-ketoester such as **97**. As in the (–)-Ptilomycalin A synthesis, this strategy is very attractive since it is highly convergent. In order for this synthesis to be completed in this convergent fashion, however, the guanidine unit would have to be introduced very early in the synthesis. Early installation of the guanidine is attractive in that further manipulations to install the guanidine at a later stage can be avoided, but since we did not want to protect the guanidine, we were forced to deal with this highly polar functionality for several steps of the synthesis (Figure 21).

Results and Discussion

Synthesis of 13,14,15-Isocrambescidin 800. The synthesis began with amine **98**, which had also been utilized in the second generation synthesis of (–)-Ptilomycalin A and the synthesis of Crambescidin 800 (See Figure 22). Treatment of **98** with 1-*H*-pyrazole-1-carboxamidine hydrochloride (Bernatowicz et al., J. Org. Chem. 1992, 57:2497-2502) and Hunig's base at 60°C afforded guanidine **99** (*ca* 99% yield) which was used without purification (Figure 22). Before examining the key Biginelli condensation, the trisubstituted olefin had to be selectively oxidized. In the event, treatment of **99** with catalytic osmium tetroxide (OsO_4) resulted in selective dihydroxylation of the trisubstituted double bond. (Sharpless and Williams, Tetrahedron Lett. The corresponding diol was cleaved with $Pb(OAc)_4$ in the presence of morpholinium acetate affording aminal **100**. Following cleavage of the diol, we found it was optimal if **100** was filtered, and used immediately in the Biginelli condensation, and not exposed to an aqueous workup. Furthermore, **100** is actually a mixture of several components as judged by ^1H and ^{13}C NMR. Multiple signals are observed for many carbon atoms in the ^{13}C NMR spectra, while broad peaks are seen in the ^1H NMR spectra and no aldehyde signal is apparent (Figure 22).

Biginelli condensation of **100** and β-ketoester **101** (Overman et al., J. Am. Chem. Soc., 1995, 117:2657-2658) in EtOH proceeded with modest selectivity (3:1 *trans*:*cis*). Fortunately, we found that heating **100** (1 equiv) and **101** (1.5 equiv) in 2,2,2-trifluoroethanol at 60°C for 20 h improved the diastereoselectivity to 7:1 *trans*:*cis*. After purification on silica gel deactivated with pH 7.0 buffer. The deactivated silica was prepared by taking Merck silica gel (0.040-0.063) adding 10% (by weight) pH 7.0 buffer and mixing until homogeneous. The desired *trans* adduct **102** was isolated in 49% yield and *cis* adduct **103** in *ca.* 5% yield. The cis adduct **103** was slower moving on silica gel than trans adduct **102**. Thus, it was somewhat difficult to isolate pure **103** since some of trans adduct **102** would trail off of the column. The stereochemistry of *trans* adduct **102** was initially assigned based on results from previous model studies (McDonald et al. J. Org. Chem 1999, 64, 1520-1528). This assignment was more rigorously established using pentacyclic intermediates (see **105a** and **105b**) produced

later in the synthesis. Deprotection of **102** with TBAF in DMF for 36 h afforded diol **104** in 80% yield (Figure 23). Often, this reaction did not go to completion, and partially deprotected intermediates (R^2 = TBS, R^3 = H) were isolated in 10–15% yield. Heating the reaction mixture at 60°C consistently afforded fully deprotected **104**, but other products were formed and the isolated yield of **104** was lower.

A. Initial Stereochemical Assignment of pentacycle 105a

Diol **104** was now properly functionalized for conversion to the isocrambescidin pentacyclic core. In the event, **104** was exposed to *p*-toluenesulfonic acid (*p*-TsOH) (3 equiv) in $CHCl_3$ for 24 h. The reaction mixture was then washed with aqueous HCO_2Na affording an approximate 1:1 mixture of the desired pentacycle **105a** and isomerized pentacycle **106** (pentacycle 20 is an approximate 1:1 mixture of diastereomers, and the structure was assigned based on ^{1}H NMR COSY studies) in *ca* 50% yield (Figure 23). Since the tosylate couterion interconverted slowly, several washings were required to obtain the formate salts exclusively, and we were concerned that the multiple washings may result in lower yields for this transformation. The formate salts were prepared to allow direct comparisons to pentacycle **107** (Figure 24), an intermediate in the first generation synthesis of (–)-Ptilomycalin A (Overman et al. J. Am. Chem. Soc. 1995, 117, 2657-2658). ^{1}H NMR NOE studies confirm that **105a** is epimeric at C13, C14 and C15 with **107**. Thus, *trans* diaxial addition of the side chain alcohol to the double bond during the conversion of **104→105a** orients the hydropyran in the Isocrambescidin core as in the Ptilomycalin A/Crambescidin core. Furthermore, that **105a** is epimeric with 13,14,15-Isocrambescidin 800 at C14 was signaled by the 11.8 Hz coupling constant of the C14 methine hydrogen (McDonald et al. J. Org. Chem 1999, 64, 1520-1528) (See Figures 23 and 24 [Scheme 3, Figure 3]).

Further studies revealed that the ratio of compounds **105a** and **106** could be controlled by varying the reaction times and equivalents of *p*-TsOH. Larger amounts of *p*-TsOH and longer reaction times favored the formation of **106**, and we found that treating pure **105a** with *p*-TsOH also afforded **106**. Conditions were found (2 equiv *p*-TsOH, $CHCl_3$, 7 h) that gave a

5:1 mixture of **105a** and **106**. Unfortunately, we could not find conditions using *p*-TsOH that gave **105a** exclusively, and since **105a** and **106** were somewhat difficult to separate, the isolated yields of **105a** were never greater than 45–50%. Thus, a better method for the preparation of **105a** was required.

Treatment of **104** with pyridinium *p*-tolunesulfonate (PPTS) (2 equiv) at 60°C in $CHCl_3$ for 5 h followed by a HCO_2Na wash afforded a 1:5 mixture of the desired **105a** and tetracycle **108a** (Figure 25). Slight modifications of the reaction conditions (2 equiv PPTS, $CHCl_3$, 90°C in a sealed tube, 24 h), however, afforded a 2:1 mixture of **105a** and **108a** (Figure 25). Unfortunately, we could not find conditions using PPTS to convert **104** completely to **105a**, but **105a** and **108a** could be separated by column chromatography. After separation, resubjection of **108a** to the reaction conditions afforded, again, a 2:1 mixture of **105a** and **108a**. The desired **105a** could be isolated in 75% combined yield after one recycle. Initially, for comparative purposes, **105a** and **108a** were converted to the formate salts before chromatography, and they were separated using 95:5:0.1 EtOAc-isopropanol-formic acid. We later found, however, that the hydrochloride salts, **105b** and **108b**, were easier to separate than the corresponding formate salts (**105a** and **108a**). The hydrochloride salts were prepared by washing the reaction mixture with 0.1 N HCl or saturated aqueous sodium chloride and separated on silica gel using 99:1 $CHCl_3$-MeOH→95:5 $CHCl_3$-MeOH. As in previous cases, several washings were required to completely convert the tosylate salt to the hydrochloride salt. Since the formate and hydrochloride salts could be obtained in virtually identical yields, the ease of separation made use of the hydrochloride salts optimal (Figure 25). Reagents used were PPTS, HCl_3, 90°C 24 h; HCO_2 Na wash or 0.1 N HCl wash ("a").

While pentacycles **105a** and **105b** could be prepared in synthetically useful yields, the sequence was somewhat cumbersome. Ideally, we needed to find conditions that did not promote the formation of isomerization products such as **106** but would afford complete conversion to **105a** or **105b**. Toward this end, we found that treatment of **104** with HCl (3 equiv) in EtOAc afforded **105b** exclusively in 78% yield. Since the hydrochloride salt was formed during the reaction, the somewhat troublesome counterion exchange was avoided

(Figure 26).

Epimerization to the axial ester at C14 was best accomplished after removal of the allyl group of the terminal ester. To this end, the allyl group in **105b** was removed with $(Ph_3P)_4Pd$ and morpholine (Figure 26). The C14 ester was then epimerized with Et_3N in MeOH at 60°C affording a 2–3:1 mixture of the desired β-epimer, **109**, and a mixture of the starting α-epimer and a product analogous to **108a** resulting from elimination of the oxygen at C15. After purification by flash chromatography, **109** was isolated in 50–60% yield for the two steps. A diagnostic 3.0 Hz coupling constant is observed for the C14 methine hydrogen of **109**. In contrast to precursors of (–)-Ptilomycalin A and Crambescidin 800, the axial ester is favored at equilibrium in the Isocrambescidin series.

The synthesis of 13,14,15-Isocrambescidin 800 was readily completed from **109** (Figure 26). The (*S*)-7-hydroxyspermidine fragment **110** was prepared from (*R*)-epichlorohydrin and coupled with pentacycle **109** using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (Castro et al. Tetrahedron Lett. 1975, 1219-22) to provide **111** in 71% yield. Removal of the BOC groups with 2 M HCl in ethyl acetate (Stahl et al. J. Org. Chem. 1978, 43, 2285-2286) and purification of the crude product by reverse-phase HPLC provided the trihydrochloride salt of 13,14,15-Isocrambescidin 800 (**10**), $[\alpha]_D^{23}$ –67.7 (*c* 0.7 MeOH), in 70% yield. The data for the trihydrochloride salt of synthetic **10** is in agreement with ^1H and ^{13}C NMR data reported for natural **10**. The ^1H NMR spectrum (500 MHz, CD3OD) of synthetic d1 trihydrochloride is identical to the spectrum of natural **10** published in the Supplementary Materials of references 3b (Spectrum S-3); Spectrum S-3 is apparently also of the tricationic salt. There are errors in the tabulated data in Table 1 of reference 3b, since there are discrepancies between the tabulated data and Spectrum S-3. The ^{13}C NMR spectrum of synthetic **10** trihydrochloride is also identical to the spectrum of natural **10** published in the Supplementary Material of reference 3b (Spectrum S-8a). Again, there are errors in the tabulated data in Table 1 of reference 3b, since there are discrepancies between the tabulated data and Spectrum S-8a. Synthetic **10** was also converted to the triacetate derivative, **112**. Data for synthetic **112** is also in agreement with ^1H and ^{13}C NMR data

reported for **112** prepared from natural **10** (Jares-Erijman, et al J. Org. Chem. 1993 58: 4805).

The trihydrochloride salt of **10** was believed to be obtained, since a basic workup was not performed after the removal of the BOC groups (**111** → **10**). Natural **10**, however, was reported with the amines as the free bases, but the ^{1}H and ^{13}C NMR spectra of synthetic **10** and natural **10** were indistinguishable. Treatment of synthetic **10** with 0.1 N NaOH saturated with NaCl resulted in downfield shifts of the C41 and C45 protons. To investigate this further, **114** was prepared from acid **113** to model the hydroxyspermidine region of 13,14,15-Isocrambescidin 800 (Figure 27). Deprotection of **114** with 2.0 M HCl in EtOAc afforded **115** in 95% yield. The chemical shifts of the C37–C45 protons were identical in **115** and **10** [C41, 2.99–2.84 ppm (m), C45, 3.14–3.08 ppm (m)] (Table 1), but the absence of the guanidine unit made the spectral analysis of **115** somewhat easier. Treatment of **115** with 0.1 N NaOH afforded **116** as the free base. There were significant upfield shifts of the C41 and C45 protons in **116** [C41, 2.66–2.58 ppm (m), C45, 2.86–2.78 ppm (m)]. This upfield shift is consistent with conversion of a hydrochloride salt to a free base. Therefore, this supports the conclusion that natural 13,14,15-Isocrambescidin 800 was actually isolated as the trihydrochloride salt (Figure 27).

TABLE 1

Comparison of the Chemical Shifts of the C41 and C45 Protons of Compounds 115 and 116

	^{1}H NMR (CD_3OD, 500 MHz), (δ ppm),mult	
Position	**115**	**116**
41	**2.99-2.84, m**	**2.6-2.58, m**
45	**3.14-3.08, m**	**2.86-2.78, m**

Assignment of the C43 Stereocenter of 13,14,15-Isocrambescidin 800. The C43 stereocenter in 13,14,15-Isocrambescidin 800 was assigned as *S* based on analogy to crambescidin 816 (compound **3**). Our completed total synthesis of compound **10** seemed to confirm this assignment, but, since the C43 stereocenter is far removed the other stereocenters, we were not certain that (43*S*)-13,14,15-Isocrambescidin 800 could be spectroscopically distinguished from (43*R*)-13,14,15-Isocrambescidin 800. To this end (43*R*)-13,14,15-Isocrambescidin 800 (**117**) was prepared from **109** and ***ent*-110.** Ent **110** was prepared from (*S*)-epichlorohydrin (Figure 28). As anticipated, **117** was indistinguishable from synthetic **10** and natural **10** by ^{1}H and ^{13}C NMR and HPLC.

To unambiguously make this stereochemical assignment, we anticipated having to compare a derivative of natural **10** to derivatives of synthetic **10** and **117**. The preparation of Mosher's derivatives of synthetic **10, 117** and natural **10** and subsequent analysis by ^{19}F NMR was an appealing option. Mosher's derivatives **118** (43*S*) and **118** (43*R*) were prepared according to the method developed by Ward (Figure 29). The corresponding Mosher's derivative of natural **10** was then prepared and compared to **118** and **119**. The ^{19}F NMR data is identical for the Mosher's derivatives prepared from natural **10** and synthetic **10**. Due to rotamers about the C38 amide, there are 6 peaks in the ^{19}F NMR spectra (See Figure 27). This unambiguously establishes that the stereochemistry at C43 in 13,14,15-Isocrambescidin 800 is *S*.

The first total synthesis of 13,14,15-Isocrambescidin 800 (**10**) was accomplished in convergent fashion with the longest linear sequence from commercially available material being 21 steps. These investigations confirm the stereochemical assignment of **10** and rigorously establish that the absolute configuration of its hydroxyspermidine side chain is *S*. In its present form, this synthesis can provide substantial quantities of **10** and congeners for pharmacological evaluation. This enantioselective total synthesis demonstrates for the first time that: (a) the tethered Biginelli strategy can be extended to guandidine intermediates, (b) the key Biginelli condensation can be accomplished under sufficiently mild conditions that fragments containing the full functionality of the Crambescidin core can be employed, and (c)

that the spiroaminal units in the Isocrambescidin series assemble with high stereochemical fidelity.

Experimental Section

General. Dry THF, Et_2O, and CH_2Cl_2 from Aldrich were filtered through a column charged with Al_2O_3 (solvent purification system). Triethylamine (Et_3N), pyridine, diisopropylethylamine, diisopropylamine, and acetonitrile were distilled from CaH_2 at atmospheric pressure. Silica gel (0.040-0.063) by Merck was used for flash chromatography. Reverse phase HPLC separations were performed using an HPLC system composed of a Waters 590 pump and a Waters 486 UV detector. NMR spectra were recorded on Bruker instruments (500 MHz and 400 MHz). IR spectra were measured on Perkin-Elmer Series 1600 FTIR, and optical rotations were measured on Jasco DIP-360 polarimeter. Mass spectra were measured on a MicroMass Analytical 7070E (CI-isobutane) or a MicroMass AutoSpec E (FAB) spectrometer. Microanalyses were performed by Atlantic Microlabs, Atlanta, GA. Other general experimental details have been described (Metais, E. et al. J. Org. Chem. 1997, 62, 9210-9216).

(6S, 11Z, 13S)-6-amino-N-carboxamidine-8-(1',3'-dioxan-2'-yl)-2-methyl-13-trisopropylsiloxypentadeca-2,11-diene (99). A solution of amine **98** (2.95 g, 6.12 mmol), 1-H-pyrazole-1-carboxamidine hydrochloride (2.70 g, 18.4 mmol), Hunig's base (4.37 mL, 24.5 mmol) and DMF (6.0 mL) was maintained at rt for 16 h then warmed to 60°C and maintained for 4 h. The solution was cooled and partitioned between $CHCl_3$ (300 mL) and 0.1 N HCl (75 mL). The organic phase was washed with 0.1 N HCl (75 mL) and H_2O (75 mL), dried (Na_2SO_4), filtered and concentrated affording a 2:1 mixture of guanidine **99** and amine **98**. This mixture was dissolved in DMF (6.0 mL) and treated with 1-H-pyrazole-1-carboxamidine hydrochloride (1.35 g, 9.2 mmol) and Hunig's base (2.19 mL, 12.3 mmol). The solution was maintained at room temperature (rt) for 16 h then warmed to 60°C and maintained for 4 h. The reaction was worked up as previously described and placed on a vacuum pump (0.1 mm Hg) to remove the residual DMF. This afforded 3.20 g (99%) of guanidine **99** as a light

yellow oil which was used without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (app d, J = 6.7 Hz, 1 H), 7.24 (br s, 1 H), 5.43-5.39 (m, 1 H), 5.29-5.24 (m, 1 H), 5.09 (br t, J = 7.0 Hz, 1 H), 4.46 (app q, J = 7.3 Hz, 1 H), 3.98-3.76 (m, 4 H), 3.60 (m, 1 H), 2.20-2.13 (m, 2 H), 2.02-1.74 (overlapping m, 2 H), 1.69 (s, 3 H), 1.64-1.58 (overlapping m, 2 H), 1.62 (s, 3 H), 1.51-1.38 (m, 2 H), 1.05 (m, 21 H), 0.87 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ157.6, 135.0, 132.7, 126.9, 123.1, 100.5, 69.8, 59.8, 59.3, 46.6, 45.0, 36.5, 31.7, 30.5, 25.7, 25.0, 24.8, 22.2, 18.1, 18.0, 17.6, 12.3, 9.3 ppm; IR (film) 2961, 2865, 1651, 1463, 1383, 1246, 1109 cm^{-1}; MS: HRMS (FAB) (M - Cl) *m/z* 524.4225, (524.4250 calcd for $C_{27}H_{58}N_3O_3Si$); $[\alpha]^{25}_D$+1.7, $[\alpha]^{25}_{577}$ +2.7, $[\alpha]^{25}_{546}$ +3.2, $[\alpha]^{25}_{435}$ +7.3, $[\alpha]^{25}_{405}$ +9.3 (*c* 1.3, $CHCl_3$).

(4aS,7S)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-3-[(4S)-4-t-butyldimethylsiloxypentyl]-7-[(5Z,7S)-2-(1',3'-dioxan-2'-yl)-7-triisopropyl-siloxy-5-nonenyl]-1,2,4a,5,6,7-hexahydro-1-imino-pyrrolo[1,2,c]-pyrimidine hydrochloride (102). 4-Methylmorpholine-N-oxide (2.16 g, 18.4 mmol) and OsO_4 (3.1 mL, 0.24 mmol, 2% in t-butanol) were added to a solution of guanidine **99** (3.2 g, ~6.1 mmol), THF (105 mL) and H_2O (15 mL). The mixture was stirred for 8 h, Florisil (1.5 g) and $NaHSO_3$ (1.5 g) were added and the resulting mixture was stirred for an additional 10 h. Celite and $MgSO_4$ were added and the mixture was filtered and the eluent was concentrated to give a brown oil. This oil was dissolved in toluene (120 mL), then morpholinium acetate (3.6g, 24.5 mmol) and $Pb(OAc)_4$ (3.3 g, 7.3 mmol) were added. The solution was maintained for 45 min, then Celite was added. The mixture was filtered through a plug of Celite, the eluent was diluted with toluene (200 mL) and this solution was concentrated to give a brown oil. The oil was azeotroped to dryness with toluene (200 mL) and the residue was combined with β-ketoester 15 (5.3 g, 9.2 mmol) and 2,2,2-trifluoroethanol (9.0 mL). This solution was maintained at 60^{0}C for 20 h and then partitioned between $CHCl_3$ (250 mL) and 0.1 N HCl (50 mL). The organic phase was washed with 0.1 N HCl (50 mL) and brine (50 mL), dried (Na_2SO_4), filtered and concentrated. ^{1}H NMR analysis revealed a 7:1 ratio of trans:cis Biginelli adducts. Purification of the crude mixture by flash chromatography ($CHCl_3$ _ 99:1 $CHCl_3$-MeOH 98:2 $CHCl_3$-MeOH) using silica gel deactivated with pH 7.0 buffer afforded 3.22 g (49%) of the desired

trans adduct, **102**, as a light brown oil and 331 mg (5%) of cis adduct **103**. Data for **102**: ^{1}H NMR (500 MHz, $CDCl_3$) δ9.06 (s, 1 H), 7.33 (s, 1 H), 5.95-5.88 (m, 1 H), 5.43 (app t, J = 9.8 Hz, 1 H), 5.31 (app dq, J = 17.2, 1.5 Hz, 1 H), 5.27-5.25 (m, 1 H), 5.23 (app dq, J = 10.4, 1.3 Hz, 1 H), 4.57 (br d, J = 5.7, 2 H), 4.46-4. 41 (m, 2 H), 4.27-4.24 (m, 1 H), 4.17-4.07 (m, 2 H), 4.01-3.95 (m, 2 H), 3.91-3.78 (m, 3 H), 2.77-2.71 (m, 2 H), 2.65-2.59 (m, 1 H), 2.45-2.40 (m, 1 H), 2.32 (t, J = 7.6 Hz, 2 H), 2.07-1.88 (m, 6 H), 1.79-1.55 (m, 11 H), 1.53-1.43 (m , 4 H), 1.31-1.25 (m, 21 H), 1.13 (d, J = 6.1 Hz, 3 H), 1.05 (s, 21 H), 0.87 (t, J = 7.4 Hz, 3 H), 0.86 (s, 9 H), 0.037 (s, 3 H), 0.032 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ173.4, 165.0, 149.9, 147.3, 135.3, 132.2, 126.4, 117.9, 100.9, 100.3, 69.8, 68.3, 64.8, 64.7, 59.9, 59.4, 57.5, 54.1, 46.1, 39.0, 34.8, 34.2, 33.3, 31.6, 30.9, 30.3, 29.6, 29.52, 29.48, 29.42, 29.3, 29.1, 29.0, 28.5, 26.0, 25.8, 24.83, 24.76, 24.4, 23.6, 22.1, 18.01, 17.98, 12.3, 9.2, -4.5, -4.7 ppm; IR (film) 2926, 2856, 1738, 1713, 1681, 1538, 1462, 1382, 1256, 1086 cm^{-1}; HRMS (FAB) (M-Cl) *m/z* 1044.6, (1044.8 calcd for $C_{59}H_{110}N_3O_8Si_2$); $[\alpha]^{25}_D$-21.2 $[\alpha]^{25}_{577}$ -21.3, $[\alpha]^{25}_{546}$ -23.3, $[\alpha]^{25}_{435}$ -28.8, $[\alpha]^{25}_{405}$ -25.1 (*c* 1.9, $CHCl_3$).

(4aS, 7S)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-7-[(5Z, 7S)-2-(1',3'-dioxan-2'-yl)-7-hydroxy-5-nonenyl]-1,2,4a,5,6,7-hexahydro-3-[(4 S)-4 -hydroxypentyl]-1-imino-pyrrolo[1,2,c]-pyrimidine hydrochloride (104). A solution of **102** (2.80 g, 2.59 mmol), TBAF (13.0 mL, 13.0 mmol, 1.0 M) and DMF (26 mL) was maintained at rt for 24 h, then more TBAF (6.0 mL, 6.0 mmol, 1.0 M) was added. The solution was maintained for 6 h then partitioned between $CHCl_3$ (200 mL) and 0.1 N HCl (75 mL). The organic phase was washed with saturated aqueous $NaHCO_2$ (2x50 mL), dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (95:5:0.1 EtOAc-isopropanol:formic acid 90:10:0.1 EtOAc-isopropanol:formic acid 85:15:0.1 EtOAc-isopropanol:formic acid) using silica gel deactivated with pH 7.0 buffer to afford the formate salt of the diol 1.68g (80%) as a light brown oil. The formate salt was easier to purify, but the chloride salt was more stable. Therefore, after purification, the formate salt was converted quantitatively to chloride salt **104** by partitioning the formate salt between $CHCl_3$ (150 mL) and 0.1 N HCl (25 mL) and washing with 0.1 N HCl (25 mL) and brine (25 mL). The organic phase was dried (Na_2SO_4), filtered and concetrated to afford diol **104**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1

H), 7.43 (s, 1 H), 5.95-5.87 (m, 1 H), 5.51-5.42 (m, 2 H), 5.31 (ddd, J = 17.2, 3.0, 1.5 Hz, 1 H), 5.22 (ddd, J = 9.2, 3.0, 1.3 Hz, 1 H), 4.57 (dt, J = 5.7, 1.3 Hz, 2 H), 4.43 (dd, J = 9.9, 4.3 Hz, 1 H), 4.32 (app q, J =7.1 Hz, 1 H), 4.28-4.25 (m, 1 H), 4.17-4.08 (m, 2 H), 4.05-3.92 (m, 3 H), 3.89-3.82 (m, 2 H), 2.91-2.86 (m, 1 H), 2.62-2.58 (m, 1 H), 2.52 (td, J = 11.8, 4.6 Hz, 1 H), 2.42-2.39 (m, 1 H), 2.32 (t, J = 7.6 Hz, 2 H), 2.16-1.96 (m, 6 H), 1.86-1.44 (m, 14 H), 1.30-1.24 (m, 22 H), 1.19 (d, J = 6.2 Hz, 3 H), 0.91 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 165.0, 149.7, 147.5, 133.5, 132.3, 130.4, 118.0, 101.0, 100.5, 68.7, 65.4, 64.85, 64.76, 60.1, 59.6, 57.6, 54.2, 45.8, 38.1, 34.7, 34.2, 33.1, 30.4, 30.2, 29.6, 29.51, 29.46, 29.37, 29.2, 29.1, 28.6, 26.0, 24.9, 24.7, 24.0, 23.5, 22.2, 9.7 ppm; IR (film) 3344, 2925, 2854, 1736, 1685, 1542, 1462, 1384, 1259, 1170, 1084, 1001 cm^{-1}; MS: HRMS (FAB) (M - Cl) *m/z* 774.5615, (774.5632 calcd for $C_{44}H_{76}N_3O_8$); $[\alpha]^{25}_D$-39.4, $[\alpha]^{25}_{577}$ -40.2, $[\alpha]^{25}_{546}$ -44.8, $[\alpha]^{25}_{435}$ -66.0, $[\alpha]^{25}_{405}$ -70.0 (*c* 1.2, $CHCl_3$).

Pentacycle 105b. Acetyl chloride (320 μL, 4.5 mmol) was added to a 0^{0}C solution of MeOH (200 μL, 5.0 mmol) and EtOAc (30 mL) to give a 0.15 M solution of HCl in EtOAc. Diol **104** (1.10 g, 1.36 mmol) was then dissolved in 27 mL of this solution (4.1 mmol of HCl). The solution was maintained at rt for 6 h, then partitioned between $CHCl_3$ (250 mL) and brine (50 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated. Purification of the residue by flash chromatography ($CHCl_3$ 99:1 $CHCl_3$-MeOH 98:2 $CHCl_3$-MeOH) gave 780 mg (78%) of pentacycle **105b** as a light yellow oil. In some instances, pentacycle **105b** was contamintated with ca. 5% of an unidentified impurity. This impurity could be removed by further purification by reverse phase HPLC, but the recovery of the desired pentacycle, **105b**, was low. Therefore, pentacycle **105b** was not purified further, and the unknown impurity was removed after the next transformation.

Data for pure **105b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.45 (br s, 1 H), 8.89 (br s, 1 H), 5.95-5.87 (m, 1 H), 5.68-5.64 (m, 1 H), 5.48 (broad d, J = 11.0 Hz, 1 H), 5.31 (app dd, J = 17.2, 1.5 Hz, 1 H), 5.23 (app dd, J = 10.4, 1.3 Hz, 1 H), 4.57 (br d, J = 5.7 Hz, 2 H), 4.51 (br d, J = 7.7 Hz, 1 H), 4.25-4.21 (m, 2 H), 4.12-4.07 (m, 1 H), 3.98-3.95 (m, 1 H), 3.77-3.72 (m, 1 H), 2.91 (d, J = 11.8 Hz, 1 H), 2.61-2.56 (m, 1 H), 2.55 (dd, J = 12.5, 2.9 Hz, 1 H), 2.32 (t, J =

7.5 Hz, 2 H), 2.30-2.28 (m, 3H), 2.21-2.17 (m, 2H), 1.91 (dd, J = 14.6, 5.3 Hz, 1 H), 1.85 (br d, J = 12.9 Hz, 1 H), 1.78-1.36 (m, 13 H), 1.32-1.20 (m, 21 H), 1.12 (d, J = 6.0 Hz, 3 H), 1.12-1.10 (m, 1 H), 0.86 (t, J = 7.3 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 169.0, 150.8, 133.3, 132.3, 129.8, 118.0, 85.5, 84.8, 70.8, 68.7, 65.5, 64.8, 58.7, 55.1, 52.1, 37.2, 37.1, 34.2, 32.9, 32.1, 30.9, 30.0, 29.6, 29.51, 29.45, 29.4, 29.2, 29.11, 29.07, 28.4, 25.9, 24.9, 23.8, 22.0, 17.9, 10.2 ppm; IR (film) 2926, 2853, 1732, 1659, 1615, 1462, 1349, 1202, 1022 cm^{-1}; HRMS (FAB) (M-Cl) *m/z* 698.5117, (698.5108 calcd for $C_{41}H_{68}N_3O_6$); $[\alpha]^{25}_D$-54.6, $[\alpha]^{25}_{577}$ -55.6, $[\alpha]^{25}_{546}$ -64.2, $[\alpha]^{25}_{435}$ -114.8, $[\alpha]^{25}_{405}$ -141.3 (*c* 1.25, $CHCl_3$).

Carboxylic Acid 109. A solution of pentacycle **105b** (50 mg, 0.068 mmol), morpholine (24 μL, 0.27 mmol), $(Ph_3P)_4Pd$ (16 mg, 0.014 mmol) and CH_3CN (5 mL) was maintained for 2 h. More morpholine (12 μL, 0.13 mmol) and $(Ph_3P)_4Pd$ (8 mg, 0.007 mmol) were added and the solution was maintained for 2 h. The solution was then partitioned between $CHCl_3$ (50 mL) and 0.1 N HCl (10 mL). The organic phase was washed with 0.1 N HCl (10 mL), dried (Na_2SO_4), filtered and concentrated to give a brown oil. The brown oil was filtered through a plug of silica gel (99:1 $CHCl_3$:MeOH 98:2 $CHCl_3$-MeOH), concentrated and dissolved in Et_3N (95 μL, 0.68 mmol) and MeOH (7 mL). The resulting solution was maintained at 60°C for 36 h then partitioned between $CHCl_3$ (50 mL) and 0.1 N HCl (8 mL). The organic phase was washed with 0.1 N HCl (8 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the crude product by flash chromatography (99:1 $CHCl_3$:MeOH 98:2 $CHCl_3$-MeOH 95:5 $CHCl_3$:MeOH) afforded 28 mg (60%) of **109** as a light yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ10.07 (br s, 1 H), 9.28 (br s, 1 H), 5.64 (app t, J = 8.1 Hz, 1 H), 5.50 (d, J =10.6 Hz, 1 H), 4.58 (br s, 1 H), 4.17-4.12 (m, 1 H), 4.02-3.97 (m, 2 H), 3.92-3.88 (m, 1 H), 3.71-3.68(m, 1 H), 3.46 (d, J = 3.0 Hz, 1 H), 2.63-2.55 (m, 1 H), 2.52 (d, J =11.0 Hz, 1 H), 2.30 (t, J = 7.4 Hz, 2 H), 2.29-2.26 (m, 1 H), 2.22-2.16 (m, 3 H), 1.85-1.80 (m, 4 H), 1.73-1.42 (m, 11 H), 1.40-1.24 (m, 23 H), 1.18 (d, J = 5.9 Hz, 3 H), 0.95 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ178.6, 167.8, 149.4, 133.7, 129.6, 85.0, 83.0, 70.7, 69.0, 65.2, 52.9, 52.1, 41.7, 37.9, 37.4, 35.1, 32.5, 31.5, 30.2, 29.7, 29.4, 29.33, 29.29, 29.19, 29.0, 28.4, 27.9, 25.7, 25.3, 24.1, 22.2, 19.7, 10.2 ppm; IR (film) 3200, 2924, 2852, 1732, 1660, 1621, 1189, 1167,

1027 cm^{-1}; HRMS (FAB) (M-Cl) *m/z* 658.4789, (658.4795 calcd for $C_{38}H_{64}N_3O_6$); $[\alpha]^{25}_D$-47.3, $[\alpha]^{25}_{577}$ -49.5, $[\alpha]^{25}_{546}$ -55.9, $[\alpha]^{25}_{435}$ -99.8, $[\alpha]^{25}_{405}$ -121.5 (*c* 1.2, $CHCl_3$).

41,45-bis-t-Butoxycarbonyl-13,14,15-Isocrambescidin 800 (111). Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (28 mg, 0.064 mmol) was added to a solution of carboxylic acid **109** (30 mg, 0.043 mmol), amine **110** (23 mg, 0.064 mmol), Et_3N (29 μL, 0.22 mmol) and CH_2Cl_2 (2.0 mL). The solution was maintained for 1 h and then was partitioned between Et_2O (40 mL) and 0.1 N HCl (10 mL). The organic phase was washed with brine (2x10 mL), dried ($MgSO_4$), filtered and concentrated to afford a crude oil. Purification of this residue by flash chromatography (99:1 $CHCl_3$-MeOH · 97:3 $CHCl_3$-MeOH) gave 32 mg (71%) of **111** as a colorless foam: ^1H NMR (500 MHz, CD_3OD) δ 5.70 (br t, *J* = 8.8 Hz, 1 H), 5.51 (d, *J* = 11.1 Hz, 1 H), 4.45 (br s, 1 H), 4.19-4.06 (m, 3 H), 3.92-3.78 (m, 3 H), 3.84 (d *J* = 3.4 Hz, 1 H), 3.59-3.23 (m, 3 H), 3.19-3.12 (m, 3 H), 3.06-2.97 (m, 2 H), 2.58 (dd, *J* = 12.8, 2.3 Hz, 1 H), 2.45-2.25 (m, 6 H), 2.18-2.12 (m, 1 H), 1.96 (dd, *J* = 13.1, 6.1 Hz, 1 H), 1.81-1.44 (m, 18 H). 1.43 (s, 18 H), 1.38-1.17 (m, 23 H), 1.16 (d, *J* = 6.0 Hz, 3 H), 0.95 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (125 MHz, CD_3OD)(the C38 amide exists as an approximate 1:1 mixture of rotamers). Some of the signals of carbons in close proximity to C38, including the carbons of the hydroxypermidine unit, are doubled. These signals are listed in parentheses: δ(176.6/176.2), 169.8, 158.6, 158.4, 150.2, 134.1, 131.3, 86.7, 84.6, 80.02, 79.96, 72.0, 70.1, (69.0/68.3), 66.2, (55.0/53.4), 54.8, 54.3, 45.0, 42.6, 39.1, (38.9/38.7), 38.1, 36.2, 34.3, 34.1, 33.7, 32.9, 31.0, 30.78, 30.75, 30.67, 30.64, 30.57, 30.54, 30.50, 30.24, 30.16, 29.7, 28.9, 28.8, 28.7, 27.0, (26.7/26.6), 25.0, 22.4, 21.0, 10.8 ppm; IR (film) 3385, 2927, 2854, 1731, 1668 (br), 1614, 1449, 1366, 1253, 1167, 1028 cm^{-1}; HRMS (FAB) *m/z* 1001.7 (M-Cl), (1001.7 calcd for $C_{55}H_{97}N_6O_{10}$). $[\alpha]^{22}_D$-68.7, $[\alpha]^{22}_{577}$ -72.9, $[\alpha]^{22}_{546}$ -83.3, $[\alpha]^{22}_{435}$ -147.7 (*c* 0.6, $CHCl_3$).

13,14,15-Isocrambescidin 800 Trihydrochloride (10). A solution of **111** (30 mg, 0.029 mmol) and 2.9 mL of a 2.0 M solution of HCl in EtOAc was maintained at rt for 30 min and then concentrated. Purification of the residue by reverse phase HPLC (3.5:1 MeOH-0.1 M NaCl, Altima C18, 5 μ column) gave 18 mg (70%) of 13,14,15-isocrambescidin 800 as its

trihydrochloride salt (a light yellow oil). Data for this sample were consistent with data published for natural **10**.

Data for synthetic **10**: ^{1}H NMR (500 MHz, CD_3OD) δ 5.70 (br t, J = 9.1 Hz, 1 H), 5.51 (br d, J = 11.2, 1 H), 4.46 (br s, 1 H), 4.18-4.06 (m, 3 H), 3.97-3.86 (m, 3 H), 3.84 (d, J = 3.3 Hz, 1 H), 3.70-3.38 (m, 3 H), 3.31-3.07 (m, 3 H), 2.99-2.84 (m, 2 H), 2.57 (dd, J = 12.9, 2.4 Hz, 1 H), 2.56-2.36 (m, 5 H), 2.31-2.24 (m, 3 H), 2.18-1.43 (m, 18 H), 1.28 (app s, 22 H), 1.16-1.15 (overlapping m, 1 H), 1.16 (d, J = 6.0 Hz, 3 H), 0.95 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, CD_3OD) (the C38 amide exists as an approximate 3:1 mixture of rotamers. Only the carbons in close proximity to C38, including some carbons of the hydroxyspermidine unit, exhibit signals due to the minor rotamer. The carbon signals of the rotamers are listed in parentheses with the major rotamer listed first: δ(177.5/176.4), 169.8, 150.2, 134.1, 131.3, 86.8, 84.6, 72.0, 70.2, (68.6/69.4), 66.2, (54.9/53.2), 54.8, 54.3, (43.9/47.9), 42.7, 39.1, 38.6 (two peaks), (38.27,38.33), 38.1, (34.2/34.0). 33.7, 32.9 (2 peaks), 31.0, 30.84, 30.80, 30.75, 30.67, 30.59, 30.54, 30.4, 30.3, 29.7, 28.9, 27.0, (26.63/26.57), 25.0, 22.5, 21.0, 10.8 ppm; MS: HRMS (FAB) *m/z* 801.6223 (M - Cl), (801.6217 calcd for $C_{45}H_{81}N_6O_6$). $[\alpha]^{22}_D$-67.7, $[\alpha]^{22}_{577}$ -70.9, $[\alpha]^{22}_{546}$ -80.6, $[\alpha]^{22}_{435}$ -147.7 (*c* 0.73, MeOH).

Peracetyl-13,14,15-Isocrambescidin 800 Hydrochloride (112). A solution of 13,14,15-isocrambescidin 800 (**1**), acetic anhydride (1.2 mL) and pyridine (2.4 mL) was maintained at rt for 20 then concentrated using a vacuum pump. The resulting residue was dissolved in $CHCl_3$ (40 mL) and washed sequentially with brine (10 mL), 0.1 N HCl (10 mL) and brine (10 mL). The solution was dried (Na_2SO_4), filtered and concentrated. Purification of the residue by flash chromatography (95:5 $CHCl_3$-MeOH) gave 8 mg (70%) of Peracetylisocrambescidin 800 (**112**). ^{1}H NMR and ^{13}C NMR data for synthetic **112** was in agreement with data reported for Peracetylioscrambescidin 800 prepared from natural 13,14,15-Isocrambescidin 800.

Data for synthetic 112: ^{1}H NMR (500 MHz, $CDCl_3$) δ10.0 (s, 1 H), 9.97 (s, 1 H of minor rotamer), 9.23 (s, 1 H), 9.19 (s, 1 H of minor rotamer), 6.86 (s, 1 H), 6.70 (s, 1 H of minor

rotamer), 6.57 (s, 1 H of minor rotamer), 6.40 (s, 1 H), 5.66 (br t, J = 8.7 Hz, 1 H), 5.50 (br d, J = 11.0 Hz, 1 H), 5.13-5. 07 (m, 1 H), 4.55 (br s, 1 H), 4.19-4.13 (m, 1 H), 4.02-3.97 (m, 2 H), 3.91-3.88 (m, 1 H), 3.72-3.69 (m, 1 H), 3.62-3.37 (m, 4 H), 3.46 (d, J = 2.8 Hz, 1 H), 3.32-3.12 (m, 3 H), 3.05-2.98 (m, 1 H), 2.55-2.52 (m, 2 H), 2.37-2.18 (m, 7 H), [2.05, 2.04, 2.01, 2.00, 1.99 (singlets of the acetate methyl groups, 9 H)], 2.00-1.37 (m, 18 H), 1.28-1.18 (m, 23 H), 1.18 (t, J = 6.0 Hz, 3 H), 0.96 (t, J = 7.3 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 173.8 (minor rotamer), 170.9, 170.8, 170.7, 167.7, 149.3, 133.6, 129.7, 85.0, 82.9, 70.8, 70.5 (minor rotamer), 65.3, 52.9, 52.1, 50.5, 48.4 (minor rotamer), 46.4 (minor rotamer), 42.6, 41.73, 41.68 (minor rotamer), 38.2, 37.4, 37.0, 36.1, 35.6, 35.4, 33.2, 33.1, 32.9, 32.3, 31.4, 30.2, 29.6, 29.5, 29.4, 29.1, 29.0, 28.5, 27.9, 27.0, 25.8, 25.5, 25.4, 24.0, 23.2, 22.1, 21.2, 20.9, 19.6, 10.2 ppm; MS: LRMS (ES) *m/z* 927.70 (M - Cl), (927.6534 calcd for $C_{51}H_{87}N_6O_9$). $[\alpha]^{25}_D$-56.1 (*c* 0.3, $CHCl_3$).

EXAMPLE V

Synthesis Plan. The structural differences and similarities between the two Crambescidin families are apparent in molecular mechanics models of the methyl esters of the 13,14,15-Isocrambescidin and Crambescidin/Ptilomycalin A pentacyclic guanidine moieties (Figure 20). The lowest energy conformation found from Monte Carlo searches using Macromodel version 5.5 and the OPLS force field is depicted (Chang, G.; Guida, W. C., Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386). Ten thousand starting conformations were examined; in all cases, several conformations that differ only in the spatial orientation of the methyl ester fragment were within a few kcals of the global minimum. For instance, the C10 and C13 angular hydrogens are trans in the isocrambescidin core and cis in the corresponding Crambescidin/Ptilomycalin A unit, while the stereochemical relationship between the substituents at C13, C14 and C15 is the same in both structures. For both alkaloid families, the C–O bonds of the hydropyran and oxepene units are axial. Thus, as in the Crambescidin/Ptilomycalin A series (Snider, B. B.; Shi, Z. *Tetrahedron Lett.* **1993**, *34*, 2099–2102; Snider, B. B.; Shi, Z. *J. Am. Chem. Soc.* **1994**, *116*, 549–557; Overman, L. E.; Rabinowitz, M. H.; Renhowe P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2657–2658;), it was

anticipated that the C8 and C15 spirocenters of the Isocrambescidins would evolve with the desired stereochemistry if the central triazaacenaphthalene ring system was constructed with the proper trans stereochemistry.

An intramolecular variant of the venerable Biginelli condensation that was introduced several years ago (Overman, L. E.; Rabinowitz, M. H. *J. Org. Chem.* **1993**, *58*, 3235–3237) has proven to be highly useful in the design of concise strategies for synthesizing complex guanidine alkaloids. As detailed in Example I, tethered Biginelli condensation of a ureido aldehyde and a β–ketoester can be employed to combine all the carbons of the Crambescidin/Ptilomycalin A pentacyclic core and set the pivotal cis relationship of the H10 and H13 hydrogens (Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937–6963). Recent exploratory studies of stereoselection in tethered Biginelli condensations were critical in the planning on how to synthesize the Isocrambescidin alkaloids (McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 1520–1528). These investigations revealed that the stereochemical outcome of tethered Biginelli condensations could be reversed if the urea component was replaced with a basic guanidine. Thus, Biginelli condensation of guanidine aldehyde (or aminal) **122** with benzyl acetoacetate provided *trans*-1-iminohexahydropyrrolo[1,2-*c*]pyrimidine **123** with high selectivity (Figure 32) (McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 1520–1528).

Based on these exploratory studies and the experience in the Crambescidin/Ptilomycalin A series, a convergent plan for preparing 13,14,15-Isocrambescidin 800 (**10**) readily emerged (Figure 33). Tethered Biginelli condensation of guanidine aldehyde **126** and β–ketoester **127** would be employed to set the critical trans C10–C13 stereorelationship and unite *all* the heavy atoms of the pentacyclic guanidine moiety. It was hoped that acid promoted dehydration of **125** would then generate the remaining three heterocyclic rings of **124** in a single step. Mindful from the outset of one challenge posed by this strategy: the guanidine functional group would be introduced early in the synthesis. Unless protection and deprotection steps are added, this highly polar functionality would be forced to carry through several stages of the synthesis.

Results and Discussion

Synthesis of trans-1-Iminohexahydropyrrolo[1,2-c]pyrimidine 134. The total syntheses of **10** and **10a** began with diene amine **128**, which was also utilized in the synthesis of (–)-Crambescidin 800 (Figure 34). Treatment of **128** with 1-*H*-pyrazole-1-carboxamidine hydrochloride (Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497–2502) and diisopropylethylamine at 60°C generated guanidine **129**, which was utilized directly without purification. The trisubstituted double bond of this intermediate next had to be cleaved to liberate the electrophilic component of the Biginelli condensation. Fortunately, the oxidation strategy that was employed to realize this degradation in the related urea series was compatible with the guanidine functionality. Thus, selective dihydroxylation of the trisubstituted double bond of **129** with catalytic osmium tetroxide (OsO_4) and *N*-methylmorpholine-*N*-oxide (NMO) (Sharpless, K. B.; Williams, D. R. *Tetrahedron Lett.* **1975**, 3045–3046), followed by cleavage of the resulting diol with $Pb(OAc)_4$ in the presence of morpholinium acetate provided **130**. This intermediate was purified only by filtration to remove PbO_2 and was a mixture of several components as judged by ^1H and ^{13}C NMR analysis. Multiple signals were observed for many carbon atoms in ^{13}C NMR spectra of **130** and ^1H NMR spectra showed several broad peaks; no aldehyde signal was apparent.

Biginelli condensation of crude **130** and β–ketoester **131** in EtOH at 60°C proceeded with modest trans selectivity (3:1). Fortunately, it was found that heating **130** with 1.5 equiv of **131** in 2,2,2-trifluoroethanol at 60°C for 20 h improved diastereoselection to 7:1. After purification of the crude products on silica gel deactivated with pH 7.0 buffer (deactivated silica was prepared by adding 10% (by weight) pH 7.0 phosphate buffer to Merck silica gel (0.040-0.063 μ) and mixing until homogeneous), the desired trans adduct **132** was isolated in 48% yield and cis adduct **133** in *ca.* 5% yield (since the cis adduct **133** was slower moving on silica gel than **132**, it was difficult to isolate pure **133**). The stereochemistry of **132** was provisionally assigned based on the earlier exploratory studies (McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 1520–1528). As seen shortly, this assignment could be confirmed

rigorously at a later stage. Deprotection of **132** with tetra-*n*-butylammonium fluoride (TBAF) in *N,N*-dimethylformamide (DMF) at room temperature for 36 h gave rise to diol **134** in 80% yield. In some runs, this reaction did not go to completion and intermediates in which only the TIPS group had been removed were isolated in 10–15% yield. Heating the reaction mixture at 60°C avoided this complication, however, other unidentified products were formed and the isolated yield of **134** was not improved.

Cyclization to Form Pentacycle 135. Initially guanidine diol **134** was exposed at room temperature to 3 equiv of *p*-toluenesulfonic acid monohydrate (*p*-TsOH•H_2O) in $CHCl_3$ for 24 h (Figure 35). After washing the reaction mixture with aqueous HCO_2Na, a 1:1 mixture of a pentacyclic product, subsequently shown to be **135a**, and tetrahydrofuryl isomer **136a** were isolated in *ca.* 50% yield (exchange of the tosylate couter ion for formate required several washings with aqueous sodium formate, which led to some erosion in yield).

The constitution of these pentacyclic products was ascertained as follows. The gross structure of pentacycle **136a**, a ~1:1 mixture of stereoisomers at the center carrying the 1-butenyl side chain, was secured by ^{1}H NMR COSY and ^{13}C NMR studies. The stereochemistry of **136a** at C15 (the crambescidin numbering system is employed in the discussion of synthetic intermediates; correct IUPAC names and numbering can be found in the Experimental Section) followed from the chemical shift of the C14 methine hydrogen (δ 2.88) (The C14 methine hydrogen of **135** is observed at δ 2.91, while this hydrogen of **139** is occurs at δ 2.30. The C15 stereochemistry of these products was rigorously determined (*vide infra*)), while the stereochemistry at C8 was not determined and is assigned on the basis of analogy only. Pentacyclic guanidines **135a** and **136a** were isolated as their formate salts to allow direct comparisons with pentacycle **137**, an intermediate in the original synthesis of (–)-Ptilomycalin A (Example II and Overman, L. E.; Rabinowitz, M. H.; Renhowe P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2657–2658). That **135a** was epimeric to **137** at C13 was signaled by the absence of an ^{1}H NMR NOE between H10 and H13 in the former, while the 11.7 Hz coupling constant of the C14 methine hydrogen of **1353a** showed that the ester side chain was equatorial.

Since none of the pentacyclic guanidine intermediates or products prepared during the investigations were crystalline, ^{1}H NMR NOE studies proved indispensable in assigning stereochemistry. A molecular mechanics model of the guanidine moiety of **135a** (an intermediate having the 13-Epicrambescidin core), which helped in analysis of critical NOE enhancements, is provided in Figure 31 (the lowest energy conformation found from Monte Carlo searches using Macromodel version 5.5 and the OPLS force field is depicted. Ten thousand starting conformations were examined; in all cases, several conformations that differ only in the spatial orientation of the methyl ester fragment were within a few kcals of the global minimum. As discussed later in the text, the conformation of the 13,15-Isocrambescidin core depicted in Figure 31 is undoubtedly not the lowest energy one). Also provided in Figure 31 are models of the two additional guanidine pentacycles (13,14,15-isocrambescidin and 13,15-epicrambescidin ring systems) and, for reference, a model of the Crambescidin/Ptilomycalin A pentacyclic guanidine moiety.

Additional investigation revealed that formation of tetrahydrofuran isomer **136a** from **134** could be controlled by varying reaction time and equivalents of *p*-TsOH•H_2O. Larger amounts of acid and longer reaction times favored the formation of **136a**. Exposing **135a** to *p*-TsOH•H_2O at room temperature for extended periods also led to **136a**. The best conditions found for generating **135a** involved exposing **134** to 2 equiv of *p*-TsOH•H_2O in $CHCl_3$ for 7 h at room temperature; a 5:1 mixture of **135a** and **136a** was produced. Since these isomers were difficult to separate, the isolated yield of **135a** produced in this way was never greater than 50%.

Pyridinium *p*-toluenesulfonate (PPTS) was examined to cleave the 1,3-dioxane protecting group of **134** and promote cyclization of the resulting keto guanidine diol. With this weaker acid, higher reaction temperatures were required and mixtures of **135a**, tetracyclic vinylogous carbamate **138a** and several unidentified minor byproducts were produced (Figure 36). When **134** was heated with 2 equiv of PPTS at 60°C in $CHCl_3$ for 5 h and the crude product was washed with aqueous HCO_2Na, **135a** and **138a** were generated in a 1:5 ratio. Increasing the

reaction temperature to 90°C (sealed tube) for 24 h provided **135a** and **138a** in a 2:1 ratio (a small amount, ~10% relative to **138a**, of the formate analog of **139** was also produced. When **138a** was heated with PPTS at 90°C, **135a** and **138a** were formed also in a ~2:1 ratio). Separation of these products on silica gel, followed by resubjection of **138a** to PPTS at 90°C gave **135a** in 75% combined yield.

Initially, **135a** and **138a** were converted to their formate salts prior to chromatography and were eluted from deactivated silica gel using 95:5:0.1 EtOAc-isopropanol-formic acid. It was later found that the hydrochloride salts, **135b** and **138b**, were easier to separate on silica gel. These salts were prepared by washing the reaction mixture with 0.1 M HCl or saturated aqueous sodium chloride; several washings were required to completely exchange the tosylate counter ion.

Since both NH hydrogens were readily apparent in ^{1}H NMR spectra of **135b**, extensive NMR studies (^{1}H COSY, HMQC, HMBC and NOESY) eventually revealed that **135b** had the13-Epicrambescidin stereochemistry (i.e., the spiro hydropyran and ester side chain are both epimeric to those of **10** and **10a**). Key findings were diagnostic ^{1}H NMR NOEs observed between N2H and H19, N2H and H17(axial), and H13 and H16(axial); see the model of the 13-Epicrambescidin core in Figure 31. It is shown in the following discussion, the stereochemistry of both the spiro hydropyran and ester side chain can be readily inverted, allowing **135b** to be a viable intermediate for accessing Isocrambescidins **10** and **10a**.

Although the procedures just described provided pentacyclic guanidine salts **135** in synthetically useful yields, these sequences were cumbersome. Ideally, it is needed to find acidic conditions for cyclizing **134** that would not promote allylic rearrangement of the C3 alcohol, yet would irreversibly transform tetracyclic vinylogous carbamate intermediate **138** to a pentacyclic guanidine isomer. It was eventually discovered that treatment of **134** with 3 equiv of HCl in EtOAc at room temperature delivered **135b** in 78% yield (Figure 37). Careful purification of the crude cyclization product by reverse phase HPLC (9:1 MeOH–0.1 M NaCl) afforded, in addition to **135b**, 5–7% of pentacyclic guanidine **139**.

That **139** was epimeric to the Isocrambescidins only at C14 (ester side chain) was apparent from ^{1}H NMR COSY, HMQC, HMBC and NOESY experiments. The stereochemistry at C15 followed directly from diagnostic ^{1}H NMR NOEs observed between N2H and the H17(axial) and N2H and H20, and the lack of NOE between N2H and H19. This NOE data is consistent with the hydropyran ring of **139** preferentially adopting a chair conformation having the methyl substituent axial (Figure 38, conformation A). This conformational preference undoubtedly derives from two factors: (1) In the alternate hydropyran chair conformer, destabilizing *syn* pentane interactions would exist between C17 and C19 of the hydropyran ring and the carbonyl carbon of the ester group; for two views of this conformation, see Figure 38, conformation B and the model of the 13,15-Epicrambescidin core in Figure 31. (2) Conformer A would be stabilized by an anomeric interaction between the hydropyran oxygen and the C15–N2 bond (Kirby, A. J. *Stereoelectronic Effects*; Oxford University Press: Oxford, 1995; pp 3–24; Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer: Berlin, **1983**; Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon: Oxford, **1983**.).

To gain more insight into the mechanism of hydropyran formation, pure **135b** was resubjected to the cyclization conditions (3 equiv HCl in EtOAc at room temperature) to yield an approximate 8–9:1 mixture of **135b** and **139** (Figure 37). That this represents an equilibrium ratio of the C15 epimers under these conditions was established by: (a) demonstrating that the 8–9:1 mixture of **135b** and **139** was unchanged when resubjected to the reaction conditions for an additional 24 h, and (b) showing that pure **139** gives an identical ratio of epimers when exposed for 24 h to 3 equiv HCl in EtOAc. Since no intermediates or by-products having the ester side chain on the β face were detected in HCl-promoted cyclization of **134**, or HCl-promoted equilibrations of the spiro hydropyran epimers, it was surmised that the equilibration of **135b** and **139** did not involve tetracyclic intermediates such as **138**. Consistent with this hypothesis, exposure of **139** to DCl in EtOAc gave an approximate 8–9:1 mixture of **135b** and **139** without incorporation of deuterium into **135b** (Figure 39). Iminium cation **140** is the likely intermediate in the equilibration of the

spiro hydropyran epimers (although not rigorously precluded, the alternate possibility that epimerization at C15 occurs by cleavage of the N2–C15 bond to form a six-membered oxocarbenium ion intermediate to be less likely). It was concluded from these studies that formation of **135b** as the major product from HCl-promoted cyclization of **134** arises from kinetically-controlled axial protonation of the vinylogous carbamate unit of **134** to generate the protio equivalent of **140**, which undergoes thermodynamically-controlled spirocyclization to generate **135b** preferentially (in our syntheses of Ptilomycalin A and Crambescidin 800, the only spirohydrofuran products formed from acid-promoted cyclization of related vinylogous carbamates have the oxygen axial. In those cases, equilibration of hydropyran epimers by a pathway related to that depicted in Figure 39 would occur at slower rates since less stable *N*-acyliminium cations would be involved).

Epimerization of 135b at C14 and C15 to Give Pentacyclic Guanidine Acid 141 and Total Synthesis of 13,14,15-isocrambescidin 657 (10a). Not long after **135a** was first prepared, it was established that exposure of this intermediate to Et_3N in hot methanol provided a pentacyclic guanidine whose stereochemistry was identical to that of 13,14,15-Isocrambescidin 800 (**10**). Although this fact was not initially appreciated, epimerization at C14 and C15 is a coupled event. This reorganization was best accomplished after removal of the allyl group of the hexadecanoate ester. To this end, the 8–9:1 mixture of **135b** and **139** resulting from HCl-promoted cyclization of **134** was deprotected with $(Ph_3P)_4Pd$ and morpholine (Figure 40). The resulting mixture of acids was then epimerized by heating in MeOH at 60°C in the presence of 10 equiv of Et_3N. Acidification of this product with 0.1 M HCl yielded a mixture of pentacyclic guanidine acids **141** and **142** and tetracyclic guanidine **143** in an approximate ratio of 10–14:1:1 (the ratio of **141** to (**142** and **143**) was determined from the crude product mixture by ^1H NMR analysis at 500 MHz. Due to the complexity of this spectrum and the presence of minor impurities, it was estimated that this ratio is only accurate to ±20%. The ratio of **142**:**143** was more difficult to ascertain, although these products appeared to be formed in similar amounts. Attempts to resolve this mixture by HPLC were unsuccessful). The pentacyclic guanidine acid resulting from deallylation of **139** was not detected. After purification by flash chromatography on silica gel, **141**, which

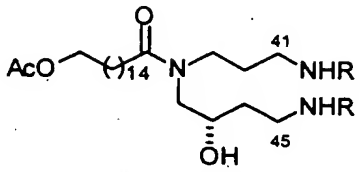
exhibits a diagnostic 3.3 Hz coupling constant for the equatorial C14 methine hydrogen, was isolated in 50–60% yield for the two steps. A similar mixture of products was obtained when pure samples of **135b** or **139** were individually deallylated and heated with Et_3N in MeOH. In contrast to precursors of (–)-Ptilomycalin A (**1**) and Crambescidin 800 (**2**) (Overman, L. E.; Rabinowitz, M. H.; Renhowe P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2657–2658), the axial ester is highly favored in the Isocrambescidin series.

The structure of **141** was secured by extensive ^1H NMR COSY, HMQC, HMBC and NOESY experiments. The stereochemistry of **141** at C15 followed from diagnostic ^1H NMR NOEs observed between H19 and H14 and between H19 and H13 (weaker), and the absence of NOEs between N2H and H19 (see the 3-dimensional model of the 13,14,15-Isocrambescidin core in Figure 2). Carboxylic acid **141** was quantitatively converted to the corresponding inner salt by washing with dilute NaOH. This product showed ^1H and ^{13}C NMR data fully consistent with those reported (Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtani, I.; Kusumi, T; Kakisawa, H. *J. Am. Chem. Soc.* **1989**, *111*, 8925–8926) for 13,14,15-Isocrambescidin 657 (**10a**). The specific rotation of synthetic **10a** was $[\alpha]^{23}_D$ –35.4 (*c* 0.8 MeOH), which agrees well with the specific rotation, $[\alpha]^{23}_D$ –32.7 (*c* 0.3 MeOH), reported (Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtani, I.; Kusumi, T; Kakisawa, H. *J. Am. Chem. Soc.* **1989**, *111*, 8925–8926) for natural 13,14,15-Isocrambescidin 657 (**10a**). Complete assignments of the ^1H and ^{13}C chemical shifts of **10a** and **141** are provided.

Since a pure sample of **138b** was available from our earlier studies of the cyclization of **134** with PPTS, this tetracyclic guanidine was deallylated to form **143** (Figure 41). Exposure of **143** to Et_3N and MeOH at 60°C provided a product mixture containing **141**, **142** and **143** in an approximate 12:1:1 ratio. As in the related conversions of **135b** and **139**, the acid congener of **139** was not detected. The experiment summarized in Figure 41 provides permissive evidence for the intermediacy of **143** in the epimerization of **135b** at C14 and C15 to provide **141**.

Total Synthesis of 13,14,15-isocrambescidin 800 (10). The (*S*)-7-hydroxyspermidine fragment **144**, which is available from (*R*)-epichlorohydrin (Coffey, D. S.; McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 8741–8742), was coupled to pentacyclic acid **141** using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219–1222) to provide **145** in 71% yield. Removal of the BOC protecting groups with 2 M HCl in ethyl acetate (Stahl, G. L.; Walter, R.; Smith, C. W. *J. Org. Chem.* **1978**, *43*, 2285–2286) and purification of the crude product by reverse-phase HPLC gave the trihydrochloride salt of 13,14,15-isocrambescidin 800 (**10**), $[\alpha]_D^{23}$ –67.7 (*c* 0.7 MeOH), in 70% yield. A specific rotation of $[\alpha]^{23}{}_D$ –48 (*c* 0.5 MeOH) is reported for natural 13,14,15-Isocrambescidin 800 (**10**) (Jares-Erijman, E. A.; Ingrum, A. L.; Carney, J. R.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 4805–4808). Since the counter ion of natural **10** was not described, the significance, if any, of this discrepancy in rotation magnitude is unknown. NMR data for the trihydrochloride salt of synthetic **10** were in good agreement with those reported for natural **10**, (Jares-Erijman, E. A.; Ingrum, A. L.; Carney, J. R.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 4805–4808). The trihydrochloride salt of **10** was obtained, since a basic workup was not performed after the removal of the BOC groups. However, natural **10** has been depicted with the spermidine nitrogens in the free base form (Berlinck, R. G. S.; Braekman, J. C.; Daloze, D.; Bruno, I.; Riccio, R.; Ferri, S.; Spampinato, S.; Speroni, E. *J. Nat. Prod.* **1993**, *56*, 1007–1015; Jares-Erijman, E. A.; Ingrum, A. L.; Carney, J. R.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 4805–4808), yet the ^1H and ^{13}C NMR spectra of synthetic **10** and natural **10** were indistinguishable. Treatment of synthetic **10** with 0.1 M NaOH saturated with NaCl resulted in downfield shifts of the C41 and C45 hydrogens. To investigate this issue further, ***i*** was prepared to model the hydroxyspermidine unit of 13,14,15-Isocrambescidin 800. Chemical shifts of the hydrogens of the hydroxyspermidine units of ***i*** and synthetic **10** were nearly identical; the absence of the guanidine unit made assignments for ***i*** straightforward. Treatment of ***i*** with 0.1 M NaOH gave ***ii*** as the free base. As summarized in the Table below, there were significant upfield shifts of the C41 and C45 hydrogens in ***ii*** upon deprotonation. This study and related experiments with synthetic **10**, provide confidence that natural 13,14,15-Isocrambescidin 800 (**10**) was isolated as the

trihydrochloride salt. Synthetic **10** was indistinguishable from a natural sample of **10** by HPLC comparisons using three eluents.



i R = H•HCl
ii R = H

^{1}H NMR shifts of the C41 and C45 hydrogens.[a]

	δ (ppm), mult	
position	*i*	*ii*
41	2.99–2.84, m	2.66–2.60, m
45	3.14–3.08, m	2.86–2.78, m

[a]In CD_3OD at 500 MHz

To provide one additional point of comparison, synthetic **10** was converted to triacetylated derivative **146**. Data for this product agreed perfectly with ^{1}H and ^{13}C NMR data reported for this derivative of natural **10** (Berlinck, R. G. S.; Braekman, J. C.; Daloze, D.; Bruno, I.; Riccio, R.; Ferri, S.; Spampinato, S.; Speroni, E. *J. Nat. Prod.* **1993**, *56*, 1007–1015).

Proof that the C43 Stereocenter of 13,14,15-Isocrambescidin 800 (10) is S. As noted earlier, the *S* configuration of the C43 stereocenter of 13,14,15-Isocrambescidin 800 (**10**) had been proposed solely by analogy with Crambescidin 816 (Berlinck, R. G. S.; Braekman, J. C.; Daloze, D.; Bruno, I.; Riccio, R.; Ferri, S.; Spampinato, S.; Speroni, E. *J. Nat. Prod.* **1993**, *56*, 1007–1015; Jares-Erijman, E. A.; Ingrum, A. L.; Carney, J. R.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 4805–4808). On the surface, our total synthesis of **10** appeared to confirm this assignment. However, since the C43 stereocenter is far removed from stereocenters of the pentacyclic guanidine moiety, it was not confident that epimers at this sterogenic center would be readily distinguished. To pursue this issue further, (43*R*)-13,14,15-Isocrambescidin 800 (**147**) was prepared from **141** and *ent*-**144** (Figure 43) (Hydroxyspermidine derivative *ent*-**144** was prepared from (*S*)-epichlorohydrin (Coffey, D.

S.; McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 8741–8742)). **147** was indistinguishable from synthetic **10** and natural **10** by ^{1}H and ^{13}C NMR comparisons as well as by HPLC analysis.

To unambiguously differentiate the C43 epimers of 13,14,15-Isocrambescidin 800, a common derivative of natural **10**, synthetic **10** and **147** were prepared. Since only 200 μg of natural **10** was available, it was chosen to employ Mosher derivatives and do the analysis by ^{19}F NMR spectroscopy (Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519). The tris Mosher derivatives **148** (43*S*) and **149** (43*R*) were prepared from (*S*)-(–)-α-methoxy–α–(trifluoromethyl)phenylacetic acid (MTPA), synthetic **10** and **147** according to the method developed by Ward (Ward, D. E.; Rhee, C. K. *Tetrahedron Lett.* **1991**, *32*, 7165–7166) and their ^{19}F NMR spectra were recorded. Since these products were mixtures of two rotamers on the NMR time scale, six ^{19}F signals were observed. Several of the signals were substantially different in diastereomers **148** and **149** (Figure 44). The (*S*)-MPTA derivatives of natural and synthetic **10** were identical, thus unambiguously establishing that the stereochemistry of 13,14,15-isocrambescidin 800 (**10**) at C43 is *S*.

Relative Energies of Pentacyclic Guanidine Stereoisomers. In contrast to the studies with Ptilomycalin A/Crambescidin compounds, the investigations with the Isocrambescidin compounds provided access to several pentacyclic guanidine stereoisomers. The relative energies of the 13,15-Epicrambescidin and 13-Epicrambescidin pentacyclic guanidine moieties are readily discerned, since **139** and **135** equilibrate at room temperature in the presence of HCl (Figure 37). No similarly clean equilibration allows us to precisely specify the relative energy of the 13,14,15-Isocrambescidin ring system. Nonetheless, that the 13,14,15-Isocrambescidin ring system is considerably more stable than the 13-Epicrambescidin guanidine moiety was signaled early in the studies when it was observed that the 13-Epicrambescidin ester **135a** was converted in good yield to the allyl ester analog of the 13,14,15-Isocrambescidin acid **141** upon treatment with Et_3N in hot methanol. Moreover, exposure of **142**, **143**, or the acid derived from **139** to methanolic Et_3N at 60°C provided the 13,14,15-Isocrambescidin acid **141** and the 13-Epicrambescidin acid **142** in an

approximate ratio of 12:1 (Figure 40). Although the complexity of this reaction mixture, the inability to isolate **142** in pure form, and analytical difficulties prevents unambiguous specification that this ratio of **141** and **142** accurately represents thermodynamic equilibrium at 60°C, this ratio is a reasonable estimate. Using this estimate, the energetic ordering of the 13-Epicrambescidin, 13,15-Epicrambescidin and 13,14,15-Isocrambescidin pentacyclic guanidine ring systems depicted in Figure 45 is obtained.

That epimerization of the 13,15-Epicrambescidin guanidine moiety at C14 would be highly favored is apparent in the molecular models shown in Figures 31 and 38. In one hydropyran chair conformer of the 13,15-Epicrambescidin ring system the ester substituent is thrust over the hydropyran ring (conformer B of Figure 38 and alternate views shown in Figure 31) and in the other chair conformer, which relieves this interaction, the methyl group is axial (conformer A of Figure 38). No such destabilizing interactions exist in the 13,14,15-Isocrambescidin ring system.

Conclusion. The first total syntheses of 13,14,15-Isocrambescidin 800 (**10**) and 13,14,15-Isocrambescidin 657 (**10a**) were accomplished in convergent fashion. The synthesis of **10** was achieved in 11% overall yield from amine **128** by a sequence involving 5 isolated intermediates. As detailed previously, **128** can be accessed from commercially available 3-butyn-1-ol in 30% overall yield by way of nine isolated and purified intermediates. Thus, the approach to the Isocrambescidins recorded herein is capable of providing these guanidine alkaloids on meaningful scales.

The total syntheses detailed herein confirm the stereochemical assignments of **10** and **10a** and rigorously establish that the absolute configuration of the hydroxyspermidine side chain of **10** is *S*. Moreover, this Example demonstrated for the first time that the tethered Biginelli strategy for preparing crambescidin alkaloids can be extended to guanidine intermediates and that the key Biginelli condensation can be accomplished under sufficiently mild conditions that fragments containing the full functionality of the Crambescidin core can be employed.

Experimental Section (Experimental details are the same as those described in the preceding example)

(6S,11Z,13S)-6-Amino-N-carboxamidine-8-(1',3'-dioxan-2'-yl)-2-methyl-13-triisopropylsiloxypentadeca-2,11-diene (129). A solution of amine **128** (2.95 g, 6.12 mmol), 1-*H*-pyrazole-1-carboxamidine hydrochloride (2.70 g, 18.4 mmol), *i*-Pr_2EtN (4.4 mL, 24 mmol) and DMF (6 mL) was maintained at rt for 16 h and then at 60°C for 4 h. The solution was cooled to rt and partitioned between $CHCl_3$ (300 mL) and 0.1 M HCl (75 mL). The organic phase was washed with 0.1 M HCl (75 mL) and H_2O (75 mL), dried (Na_2SO_4), filtered and concentrated to give a 2:1 mixture of guanidine **129** and amine **128**. This mixture was dissolved in DMF (6 mL) and again allowed to react (rt for 16 h and 60°C for 4 h) with 1-*H*-pyrazole-1-carboxamidine hydrochloride (1.35 g, 9.2 mmol) and *i*-Pr_2EtN base (2.2 mL, 12 mmol). The reaction was worked up as previously described, residual DMF was removed by evacuation for several hours at 0.1 mm to provide 3.20 g (~99%) of crude guanidine **129** as a light yellow oil. This intermediate was used without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (app d, J = 6.7 Hz, 1H), 7.24 (br s, 1H), 5.43–5.39 (m, 1H), 5.29–5.24 (m, 1H), 5.09 (br t, J = 7.0 Hz, 1H), 4.45 (app q, J = 7.3 Hz, 1H), 3.98–3.76 (m, 4H), 3.62–3.59 (m, 1H), 2.20-2.13 (m, 2H), 2.02–1.74 (overlapping m, 6H), 1.74–1.67 (m, 2H), 1.69 (s, 3H), 1.64–1.58 (overlapping m, 2H), 1.62 (s, 3H), 1.51–1.38 (m, 2H), 1.05 (m, 21H), 0.87 (t, J = 7.4 Hz, 3H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.6, 135.0, 132.7, 126.9, 123.1, 100.5, 69.8, 59.8, 59.3, 46.6, 45.0, 36.5, 31.7, 30.5, 25.7, 25.0, 24.8, 22.2, 18.1, 18.0, 17.6, 12.3, 9.3 ppm; IR (film) 2961, 2865, 1651, 1463, 1383, 1246, 1109 cm^{-1}; HRMS (FAB) *m/z* 524.4225 (524.4250 calcd for $C_{27}H_{58}N_3O_3Si$, M–Cl); $[\alpha]^{25}_D$□+1.7, $[\alpha]^{25}_{577}$ +2.7, $[\alpha]^{25}_{546}$ +3.2, $[\alpha]^{25}_{435}$ +7.3, $[\alpha]^{25}_{405}$ +9.3 (*c* 1.3, $CHCl_3$).

(4aS,7S)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-3-[(4S)-4-t-butyldimethylsiloxypentyl]-7-[(5Z,7S)-2-(1',3'-dioxan-2'-yl)-7-triisopropylsiloxy-5-nonenyl]-1,2,4a,5,6,7-hexahydro-1-imino-pyrrolo[1,2-c]-pyrimidine Hydrochloride (132). *N*-Methylmorpholine-*N*-oxide (2.16 g, 18.4 mmol) and OsO_4 (3.1 mL, 0.24 mmol, 2% in *tert*-butanol) were added to a solution of guanidine **129** (3.2 g, ~6.1 mmol), THF (105 mL) and

H_2O (15 mL). The mixture was stirred at rt for 8 h, Florisil (1.5 g) and $NaHSO_3$ (1.5 g) were added, and the resulting mixture was stirred for an additional 10 h. Celite and $MgSO_4$ then were added, the mixture was filtered and the eluent was concentrated to give the corresponding crude diol as a brown oil. This oil was dissolved in toluene (120 mL) and morpholinium acetate (3.6 g, 24 mmol) and $Pb(OAc)_4$ (3.3 g, 7.3 mmol) were added. The resulting mixture was maintained at rt for 45 min and Celite was added. This mixture was filtered through a plug of Celite, the eluent was diluted with toluene (200 mL) and the solution was concentrated to give a brown oil. This oil was azeotroped to dryness with toluene (200 mL) and the residue was combined with β–ketoester **131** (5.3 g, 9.2 mmol) and 2,2,2-trifluoroethanol (9 mL). The resulting solution was maintained at 60°C for 20 h and then partitioned between $CHCl_3$ (250 mL) and 0.1 M HCl (50 mL). The organic phase was washed with 0.1 M HCl (50 mL) and brine (50 mL), dried (Na_2SO_4), filtered and concentrated. Analysis by ^{1}H NMR revealed a 7:1 ratio of *trans*:*cis* Biginelli adducts. Purification of the crude mixture by flash chromatography ($CHCl_3$ → 99:1 $CHCl_3$-MeOH → 98:2 $CHCl_3$–MeOH) on silica gel deactivated with pH 7.0 buffer (McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 1520–1528) provided 3.22 g (48% from **128**) of the desired *anti* adduct **132** as a light brown oil and 331 mg (5% from **128**) of *syn* adduct **133**. Data for **132**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.33 (s, 1H), 5.95–5.88 (m, 1H), 5.43 (app t, J = 9.8 Hz, 1H), 5.31 (app dq, J = 17.2, 1.5 Hz, 1H), 5.27–5.25 (m, 1H), 5.23 (app dq, J = 10.4, 1.3 Hz, 1H), 4.57 (br d, J = 5.7, 2H), 4.46–4.41 (m, 2H), 4.27–4.24 (m, 1H), 4.17–4.07 (m, 2H), 4.01–3.95 (m, 2H), 3.91–3.78 (m, 3H), 2.77–2.71 (m, 2H), 2.65–2.59 (m, 1H), 2.45–2.40 (m, 1H), 2.32 (t, J = 7.6 Hz, 2H), 2.07–1.88 (m, 6H), 1.79–1.55 (m, 11H), 1.53–1.43 (m, 4H), 1.31–1.25 (m, 21H), 1.13 (d, J = 6.1 Hz, 3H), 1.05 (s, 21H), 0.87 (t, J = 7.4 Hz, 3H), 0.86 (s, 9H), 0.037 (s, 3H), 0.032 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 165.0, 149.9, 147.3, 135.3, 132.2, 126.4, 117.9, 100.9, 100.3, 69.8, 68.3, 64.8, 64.7, 59.9, 59.4, 57.5, 54.1, 46.1, 39.0, 34.8, 34.2, 33.3, 31.6, 30.9, 30.3, 29.6, 29.52, 29.48, 29.42, 29.3, 29.1, 29.0, 28.5, 26.0, 25.8, 24.83, 24.76, 24.4, 23.6, 22.1, 18.01, 17.98, 12.3, 9.2, –4.5, –4.7 ppm; IR (film) 2926, 2856, 1738, 1713, 1681, 1538, 1462, 1382, 1256, 1086 cm^{-1}; HRMS (FAB) *m/z* 1044.6 (1044.8 calcd for $C_{59}H_{110}N_3O_8Si_2$ M–Cl); $[\alpha]^{25}_D$ –21.2, $[\alpha]^{25}_{577}$ –21.3, $[\alpha]^{25}_{546}$ –23.3, $[\alpha]^{25}_{435}$ –28.8, $[\alpha]^{25}_{405}$ –25.1 (*c* 1.9, $CHCl_3$).

(4aS,7S)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-7-[(5Z,7S)-2-(1',3'-dioxan-2'-yl)-7-hydroxy-5-nonenyl]-1,2,4a,5,6,7-hexahydro-3-[(4S)-4-hydroxypentyl]-1-iminopyrrolo[1,2-c]pyrimidine Hydrochloride (134). A solution of **132** (2.80 g, 2.59 mmol), tetrabutylammonium fluoride (TBAF, 13 mL, 13 mmol, 1.0 M) and DMF (26 mL) was maintained at rt for 24 h, then more TBAF (6 mL, 6 mmol, 1.0 M) was added. The solution was maintained at for 6 h then partitioned between $CHCl_3$ (200 mL) and 0.1 M HCl (75 mL). The organic phase was washed with saturated aqueous HCO_2Na (2 × 50 mL), dried (Na_2SO_4), filtered and the filtrate was concentrated. The crude product was purified by flash chromatography (95:5:0.1 EtOAc–isopropanol–formic acid → 90:10:0.1 EtOAc–isopropanol–formic acid → 85:15:0.1 EtOAc–isopropanol–formic acid) on silica gel deactivated with pH 7.0 buffer to give the formate salt of the diol 1.68 g (80%) as a light brown oil.

The formate salt was easier to purify, but the chloride salt was more stable. Therefore, after purification, the formate salt was converted quantitatively to chloride salt **134** by partitioning the formate salt between $CHCl_3$ (150 mL) and 0.1 M HCl (25 mL) and washing the organic layer with 0.1 M HCl (25 mL) and brine (25 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated to give diol **134**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.43 (s, 1H), 5.95–5.87 (m, 1H), 5.51–5.42 (m, 2H), 5.31 (ddd, J = 17.2, 3.0, 1.5 Hz, 1H), 5.22 (ddd, J = 9.2, 3.0, 1.3 Hz, 1H), 4.57 (dt, J = 5.7, 1.3 Hz, 2H), 4.43 (dd, J = 9.9, 4.3 Hz, 1H), 4.32 (app q, J = 7.1 Hz, 1H), 4.28–4.25 (m, 1H), 4.17–4.08 (m, 2H), 4.05–3.92 (m, 3H), 3.89–3.82 (m, 2H), 2.91–2.86 (m, 1H), 2.62–2.58 (m, 1H), 2.52 (dt, J = 11.8, 4.6 Hz, 1H), 2.42–2.39 (m, 1H), 2.32 (t, J = 7.6 Hz, 2H), 2.16–1.96 (m, 6H), 1.86–1.72 (m, 3H), 1.70–1.44 (m, 11H), 1.30–1.24 (m, 22H), 1.19 (d, J = 6.2 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 165.0, 149.7, 147.5, 133.5, 132.3, 130.4, 118.0, 101.0, 100.5, 68.7, 65.4, 64.85, 64.76, 60.1, 59.6, 57.6, 54.2, 45.8, 38.1, 34.7, 34.2, 33.1, 30.4, 30.2, 29.6, 29.51, 29.46, 29.37, 29.2, 29.1, 28.6, 26.0, 24.9, 24.7, 24.0, 23.5, 22.2, 9.7 ppm; IR (film) 3344, 2925, 2854, 1736, 1685, 1542, 1462, 1384, 1259, 1170, 1084, 1001 cm^{-1}; MS: HRMS (FAB) *m/z* 774.5615 (774.5632 calcd for $C_{44}H_{76}N_3O_8$, M–Cl); $[\alpha]^{25}_D$ –39.4, $[\alpha]^{25}_{577}$ –40.2,

$[\alpha]^{25}_{546}$ –44.8, $[\alpha]^{25}_{435}$ –66.0, $[\alpha]^{25}_{405}$ –70.0 (*c* 1.2, $CHCl_3$).

Formation of Pentacycle 19b from 18 by Reaction with Methanolic HCl. Acetyl chloride (320 μL, 4.5 mmol) was added to a 0°C solution of MeOH (200 μL, 5.0 mmol) and EtOAc (30 mL) to give a 0.15 M solution of HCl in EtOAc. Diol **134** (1.10 g, 1.36 mmol) was then dissolved in 27 mL of this solution. This solution (containing 4.1 mmol of HCl) was maintained at rt for 6 h, then partitioned between $CHCl_3$ (250 mL) and brine (50 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated. Purification of the residue by flash chromatography ($CHCl_3$ → 99:1 $CHCl_3$–MeOH → 98:2 $CHCl_3$–MeOH) gave 780 mg (78%) of an approximate 8–9:1 mixture of pentacycles **135b** and **139** as a light yellow oil (it was difficult to measure accurately the ratio of **135b** and **139**, since many peaks in the ^1H NMR spectra overlapped). This mixture was used without further purification in the next step.

For characterization purposes, a sample of this mixture was purified by reverse phase HPLC (9:1 MeOH–0.1 M NaCl). To insure that the counterions of **135b** and **139** were uniquely chloride, pure samples of **135b** and **139** were dissolved in $CHCl_3$ (50 mL), washed with 0.1 M HCl (10 mL), and the organic phases were dried (Na_2SO_4), filtered and concentrated (there are small differences in the ^1H NMR and ^{13}C NMR spectra of **135b**, **139** and **10a** before and after washing with 0.1 M HCl. In Example IV, **135b** and **10a** were not washed with 0.1 M HCl after purification.

Data for **135b**: ^1H NMR (500 MHz, $CDCl_3$) δ 10.37 (s, 1H), 9.81 (s, 1H), 5.95–5.87 (m, 1H), 5.69–5.65 (m, 1H), 5.48 (br d, J = 10.9 Hz, 1H), 5.31 (dq, J = 17.2, 1.5 Hz, 1H), 5.22 (dq, J = 10.4, 1.3 Hz, 1H), 4.57 (dt, J = 5.7, 1.4 Hz, 2H), 4.50 (br d, J = 8.1 Hz, 1H), 4.31–4.27 (m, 1H), 4.26–4.21 (m, 1H), 4.12–4.07 (m, 1H), 3.98–3.95 (m, 1H), 3.77–3.72 (m, 1H), 2.91 (d, J = 11.7 Hz, 1H), 2.58–2.53 (m, 2H), 2.32 (t, J = 7.6 Hz, 2H), 2.31–2.28 (m, 3H), 2.21–2.17 (m, 2H), 1.93 (dd, J = 14.5, 5.3 Hz, 1H), 1.86–1.72 (m, 3H), 1.69–1.60 (m, 7H), 1.57–1.36 (m, 6H), 1.32–1.20 (m, 19H), 1.17–1.12 (m, 1H), 1.13 (d, J = 6.0 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 169.0, 150.9, 133.3, 132.3, 129.8, 118.0, 85.6,

84.7, 70.8, 68.8, 65.5, 64.8, 58.5, 55.1, 52.2, 37.5, 37.2, 34.2, 33.0, 32.1, 30.9, 30.0, 29.56, 29.53, 29.46, 29.38, 29.2, 29.11, 29.09, 28.5, 25.9, 24.9, 23.8, 22.0, 18.0, 10.2 ppm; IR (film) 2926, 2853, 1732, 1659, 1615, 1462, 1349, 1202, 1022 cm^{-1}; HRMS (FAB) *m/z* 698.5117 (698.5108 calcd for $C_{41}H_{68}N_3O_6$, M–Cl); $[\alpha]^{25}_D$ –54.6, $[\alpha]^{25}_{577}$ –55.6, $[\alpha]^{25}_{546}$ –64.2, $[\alpha]^{25}_{435}$ –115, $[\alpha]^{25}_{405}$ –141 (*c* 1.25, $CHCl_3$).

Data for minor pentacycle **139**: ^1H NMR (500 MHz, $CDCl_3$) δ 10.23 (s, 1H), 9.59 (s, 1H), 5.96–5.88 (m, 1H), 5.68–5.64 (m, 1H), 5.48 (br d, *J* = 11.0 Hz, 1H), 5.31 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.23 (dq, *J* = 10.4, 1.3 Hz, 1H), 4.57 (dt, *J* = 5.7, 1.3 Hz, 2H), 4.56 (br s, 1H), 4.16 (t, *J* = 6.7 Hz, 2H), 4.08 (dt, *J* = 11.0, 5.4 Hz, 1H), 3.97–3.92 (m, 1H), 3.91–3.88 (m, 1H), 2.57–2.52 (m, 2H), 2.46–2.43 (m, 2H), 2.33 (t, *J* = 7.5 Hz, 2H), 2.30 (d, *J* = 11.1 Hz, 1H), 2.30–2.26 (m, 1H), 2.25–2.17 (m, 2H), 1.92 (dd, *J* = 14.2, 5.8 Hz, 1H), 1.77–1.42 (m, 16H), 1.36 (t, *J* = 12.3 Hz, 1H), 1.33 (d, *J* = 6.7 Hz, 3H), 1.32–1.24 (m, 19H), 0.85 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 167.9, 148.9, 133.3, 132.3, 129.8, 118.1, 84.7, 82.9, 70.7, 70.1, 65.6, 64.9, 54.6, 53.0, 52.5, 37.8, 36.8, 34.2, 31.1, 30.33, 30.31, 29.61, 26.56, 29.49, 29.42, 29.23, 29.15, 29.11, 28.6, 28.4, 25.9, 24.9, 23.9, 21.8, 14.1, 10.3 ppm; IR (film) 2926, 2853, 1732, 1662, 1620 cm^{-1}; LRMS (FAB) *m/z* 698.51 (698.5108 calcd for $C_{41}H_{68}N_3O_6$ M–Cl); $[\alpha]^{25}_D$ –73.2, $[\alpha]^{25}_{577}$ –67.3, $[\alpha]^{25}_{546}$ –81.5, $[\alpha]^{25}_{435}$ –149, $[\alpha]^{25}_{405}$ –184 (*c* 0.3, $CHCl_3$).

Carboxylic Acid 25 and 13,14,15-Isocrambescidin 657 (10a). A solution of the 8–9:1 mixture of **135b** and **139** (50 mg, 0.068 mmol), morpholine (24 μL, 0.27 mmol), $(Ph_3P)_4Pd$ (16 mg, 0.014 mmol) and MeCN (5 mL) was maintained at rt for 2 h. Additional morpholine (12 μL, 0.13 mmol) and $(Ph_3P)_4Pd$ (8 mg, 0.007 mmol) were added and the solution was maintained at rt for an additional 2 h. The solution was then partitioned between $CHCl_3$ (50 mL) and 0.1 M HCl (10 mL). The organic phase was washed with 0.1 M HCl (10 mL), dried (Na_2SO_4), filtered and concentrated to give a brown oil. The brown oil was filtered through a plug of silica gel (99:1 $CHCl_3$–MeOH → 98:2 $CHCl_3$–MeOH), concentrated and the residue was dissolved in Et_3N (95 μL, 0.68 mmol) and MeOH (7 mL). The resulting solution was maintained at 60°C for 36 h and then partitioned between $CHCl_3$ (50 mL) and 0.1 M HCl (8

mL). The organic phase was washed with 0.1 M HCl (8 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the residue by flash chromatography (99:1 $CHCl_3$–MeOH → 98:2 $CHCl_3$–MeOH → 95:5 $CHCl_3$–MeOH) provided 28 mg (60%) of **141** as a light yellow oil. To insure that the counterion was uniquely chloride, **141** was dissolved in $CHCl_3$ (50 mL) and washed with 0.1 M HCl (10 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated. Data for **141**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.00 (s, 1H), 9.23 (s, 1H), 5.64 (app t, J = 8.1 Hz, 1H), 5.50 (br d, J = 11.0 Hz, 1H), 4.57 (br s, 1H), 4.16–4.11 (m, 1H), 4.03–3.99 (m, 1H), 4.00–3.97 (m, 1H), 3.92–3.88 (m, 1H), 3.72–3.68 (m, 1H), 3.45 (d, J = 3.3 Hz, 1H), 2.59–2.51 (m, 2H), 2.33 (t, J = 7.5 Hz, 2H), 2.29–2.24 (m, 1H), 2.24–2.17 (m, 3H), 1.89–1.80 (m, 4H), 1.75–1.45 (m, 10H), 1.39 (t, J = 12.3 Hz, 1H), 1.30–1.24 (m, 23H), 1.18 (d, J = 6.0 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.4, 167.7, 149.3, 133.6, 129.6, 85.0, 82.9, 70.8, 69.1, 65.3, 52.8, 52.0, 41.7, 38.1, 37.4, 33.9, 32.7, 31.4, 30.2, 29.5, 29.43, 29.37, 29.35, 29.2, 29.1, 29.0, 28.5, 27.9, 25.8, 24.7, 24.0, 22.1, 20.0, 10.2 ppm; IR (film) 3200, 2924, 2852, 1732, 1660, 1621, 1189, 1167, 1027 cm^{-1}; HRMS (FAB) *m/z* 658.4789, (658.4795 calcd for $C_{38}H_{64}N_3O_6$, M–Cl); $[\alpha]^{25}_D$□–47.3, $[\alpha]^{25}_{577}$ –49.5, $[\alpha]^{25}_{546}$ –55.9, $[\alpha]^{25}_{435}$ –99.8, $[\alpha]^{25}_{405}$ –122 (*c* 1.2, $CHCl_3$).

Carboxylic acid **141** was quantitatively converted to the carboxylate inner salt by washing a $CHCl_3$ (5 mL) solution of the acid (5 mg) with 1 M NaOH (1 mL) and brine (1 mL). The organic layer was dried (Na_2SO_4) and then concentrated to provide **10a** as a colorless oil: $[\alpha]^{25}_D$ –35.4 (*c* 0.8, MeOH). Spectroscopic and mass spectrometric data for this sample were consistent with data published for natural **10a**.

41,45-Di-tert-butoxycarbonyl-13,14,15-isocrambescidin 800 (145). A solution of carboxylic acid **141** (30 mg, 0.043 mmol), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (28 mg, 0.064 mmol), (*S*)-hydroxyspermidine derivative **144** (23 mg, 0.064 mmol), Et_3N (29 μL, 0.22 mmol) and CH_2Cl_2 (2.0 mL) was maintained at rt for 1 h and then partitioned between Et_2O (40 mL) and 0.1 M HCl (10 mL). The organic phase was washed with brine (2 × 10 mL), dried ($MgSO_4$), filtered and concentrated. Purification of this residue by flash chromatography (99:1 $CHCl_3$–MeOH → 97:3 $CHCl_3$–MeOH) gave 32 mg

(71%) of **145** as a colorless foam: ^{1}H NMR (500 MHz, CD_3OD) δ 5.70 (br t, J = 8.8 Hz, 1H), 5.51 (d, J = 11.1 Hz, 1H), 4.45 (br s, 1H), 4.19–4.06 (m, 3H), 3.92–3.78 (m, 3H), 3.84 (d J = 3.4 Hz, 1H), 3.59–3.23 (m, 3H), 3.19–3.12 (m, 3H), 3.06–2.97 (m, 2H), 2.58 (dd, J = 12.8, 2.3 Hz, 1H), 2.45–2.32 (m, 4H), 2.31–2.24 (m, 2H), 2.18–2.12 (m, 1H), 1.96 (dd, J = 13.1, 6.1 Hz, 1H), 1.81–1.44 (m, 18H). 1.43 (s, 18H), 1.38–1.17 (m, 23H), 1.16 (d, J = 6.0 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) (The C38 amide exists on the NMR time scale as an approximate 1:1 mixture of rotamers. Some of the signals of carbons in close proximity to C38, including the carbons of the hydroxyspermidine unit, are doubled. In cases where the rotamers can be distinguished, these signals are listed in parentheses) δ (176.6/176.2), 169.8, 158.6, 158.4, 150.2, 134.1, 131.3, 86.7, 84.6, 80.02, 79.95, 72.0, 70.1, (69.0/68.3), 66.2, (55.0/53.4), 54.8, 54.3, 45.0, 42.6, 39.1, (38.9/38.7), 38.1, 36.2, 34.3, 34.1, 33.7, 32.9, 31.0, 30.78, 30.75, 30.67, 30.64, 30.57, 30.54, 30.50, 30.24, 30.16, 29.7, 28.9, 28.8, 28.7, 27.0, (26.7/26.6), 25.0, 22.4, 21.0, 10.8 ppm; IR (film) 3385, 2927, 2854, 1731, 1668 (br), 1614, 1449, 1366, 1253, 1167, 1028 cm^{-1}; HRMS (FAB) *m/z* 1001.7 (1001.7 calcd for $C_{55}H_{97}N_6O_{10}$, M–Cl); $[\alpha]^{22}_D$□–68.7, $[\alpha]^{22}_{577}$ –72.9, $[\alpha]^{22}_{546}$ –83.3, $[\alpha]^{22}_{435}$ –148 (*c* 0.6, $CHCl_3$).

13,14,15-Isocrambescidin 800 Trihydrochloride (10). A solution of **145** (30 mg, 0.029 mmol) and 2.9 mL of a 2.0 M solution of HCl in EtOAc was maintained at rt for 30 min and then concentrated. Purification of the residue by reverse phase HPLC (3.5:1 MeOH–0.1 M NaCl, 5 μ Altima C18 column) gave 18 mg (70%) of 13,14,15-Isocrambescidin 800 (**10**), a light yellow oil, as its trihydrochloride salt: $[\alpha]^{22}_D$□–67.7, $[\alpha]^{22}_{577}$ –70.9, $[\alpha]^{22}_{546}$ –80.6 (*c* 0.73, MeOH). NMR data for this sample were consistent with data published for natural **10** and synthetic **10** was indistinguishable from a natural sample of **10** by HPLC comparisons using three eluents.

Preparation of Peracetyl-13,14,15-isocrambescidin 800 Hydrochloride (146). A solution of 13,14,15-isocrambescidin 800 (**10**), acetic anhydride (1.2 mL) and pyridine (2.4 mL) was maintained at rt for 20 h then concentrated using a vacuum pump. The residue was dissolved in $CHCl_3$ (40 mL) and washed sequentially with brine (10 mL), 0.1 M HCl (10 mL) and brine

(10 mL). The solution was dried (Na_2SO_4), filtered and concentrated. Purification of the residue by flash chromatography (95:5 $CHCl_3$–MeOH) gave 8 mg (70%) of peracetylisocrambescidin 800 (**146**). ^1H NMR and ^{13}C NMR data for synthetic **146** were in perfect agreement with data reported for this derivative of natural 13,14,15-Isocrambescidin 800 (Berlinck, R. G. S.; Braekman, J. C.; Daloze, D.; Bruno, I.; Riccio, R.; Ferri, S.; Spampinato, S.; Speroni, E. *J. Nat. Prod.* **1993**, *56*, 1007–1015).

(4aR,7S)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-3-[(4S)-4-t-butyldimethylsiloxypentyl]-7-[(5Z,7S)-2-(1',3'-dioxan-2'-yl)-7-triisopropyl-siloxy-5-nonenyl]-1,2,4a,5,6,7-hexahydro-1-iminopyrrolo[1,2,c]-pyrimidine Hydrochloride (133). ^1H NMR (500 MHz, $CDCl_3$) δ 9.16 (s, 1H), 6.99 (s, 1H), 5.94–5.87 (m, 1H), 5.42 (br t, J = 9.8 Hz, 1H), 5.30 (dq, J = 17.2, 1.5 Hz, 1H), 5.27–5.24 (m, 1H), 5.22 (dq, J = 10.4, 1.3 Hz, 1H), 4.56 (dt, J = 5.6, 1.4 Hz, 2H), 4.46–4.41 (m, 2H), 4.24–4.21 (m, 1H), 4.18–4.08 (m, 2H), 4.04–3.89 (m, 5H), 2.82–2.77 (m, 1H), 2.66–2.57 (m, 2H), 2.32 (t, J = 7.6 Hz, 2H), 2.27–2.19 (m, 1H), 2.03–1.55 (m, 17H), 1.31–1.24 (m, 25H), 1.12 (d, J = 6.0 Hz, 3H), 1.04 (s, 21H), 0.87 (t, J = 7.6 Hz, 3H), 0.85 (s, 9H), 0.028 (s, 3H), 0.024 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 164.8, 150.7, 149.6, 135.2, 132.2, 126.5, 117.9, 102.8, 100.0, 69.8, 69.7, 68.2, 64.8, 60.0, 59.4, 57.8, 52.2, 44.4, 39.0, 34.2, 33.5, 31.6, 30.8, 30.1, 30.0, 29.54, 29.52, 29.48, 29.41, 29.3, 29.1, 29.0, 28.5, 26.0, 25.8, 24.8, 24.4, 23.5, 22.1, 18.01, 17.99, 12.3, 9.2, –4.6, –4.7 ppm; MS (FAB) *m/z* 1044.3 (1044.8 calcd for $C_{59}H_{110}N_3O_8Si_2$, M–Cl).

Tetracyclic Guanidine 138b. ^1H NMR (500 MHz, $CDCl_3$) δ 10.46, (s, 1H), 5.94 –5.87 (m, 1H), 5.67–5.64 (m, 1H), 5.48 (br d, J = 10.9 Hz, 1H), 5.30 (dq, J = 17.2, 1.5 Hz, 1H), 5.22 (dq, J = 10.7, 1.3 Hz, 1H), 4.56 (dt, J = 5.7, 1.3 Hz, 2H), 4.56 (br s, 1H), 4.20–4.08 (m, 3H), 4.05–3.99 (m, 1H), 3.94–3.91 (m, 1H), 3.68 (br s, 1H), 2.99–2.94 (m, 1H), 2.70–2.58 (m, 3H), 2.52–2.45 (m, 1H), 2.39–2.30 (m, 3H), 2.32 (t, J = 7.6 Hz, 2H), 2.24–2.19 (m, 1H), 2.04–1.99 (m, 1H), 1.91 (dd, J = 14.8, 5.2 Hz, 1H), 1.87–1.71 (m, 4H), 1.68–1.58 (m, 5H), 1.57–1.40 (m, 4H), 1.38–1.23 (m, 20H), 1.21 (d, J = 6.2 Hz, 3H), 0.84 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 164.4, 151.4, 149.4, 133.2, 132.3, 129.7, 118.0, 104.0, 85.1, 71.3, 65.9, 65.0, 64.9, 55.6, 51.8, 37.7, 37.0, 36.4, 34.2, 31.7, 31.3, 30.2, 29.6, 29.54,

29.48, 29.40, 29.2, 29.1, 28.6, 26.1, 24.9, 24.4, 24.2, 23.6, 10.4 ppm; IR (film) 3372, 2925, 1737, 1689, 1651, 1613, 1547, 1455, 1341 cm^{-1}; MS (FAB) *m/z* 698.5106 (698.5108 calcd for $C_{41}H_{68}N_3O_6$, M–Cl).

N-Acylated Hydroxyspermidine Hydrochloride Salt i. ^1H NMR (500 MHz, CD_3OD) δ 4.04 (t, J = 6.7 Hz, 2H), 3.97–3.95 (m, 1H), 3.69–3.38 (m, 3H), 3.32–3.21 (m, 1H), 3.14–3.08 (m, 2H), 2.99–2.84 (m, 2H), 2.54–2.39 (m, 2H), 2.01 (s, 3H), 2.00–1.80 (m, 3H), 1.74–1.71 (m, 1H), 1.63–1.58 (m, 4H), 1.33–1.29 (m 22H); ^{13}C NMR (125 MHz, CD_3OD) (The amide exists as an approximate 3:1 mixture of rotamers on the NMR time scale. Carbons in close proximity to the amide, including some carbons of the hydroxyspermidine unit, exhibit two signals. In cases where two rotamers were observed, carbon signals of the rotamers are listed in parentheses with the major rotamer listed first) δ (177.5, 176.4), 173.0, (68.6, 69.4), 65.7, (54.8, 53.2), (43.9, 47.8), 38.5, (38.2, 38.3), 34.2, 34.0, (32.9, 33.0), 30.75, 30.72, 30.67, 30.61, 30.5, 30.3, 29.7, 27.0, (26.61, 27.8), (26.54, 26.59), 20.8 ppm.

N-Acylated Hydroxyspermidine Free Base ii. ^1H NMR (500 MHz, CD_3OD) δ 4.04 (t, J = 6.7 Hz, 2H), 3.91–3.85 (m, 1H), 3.65–3.32 (m, 3H), 3.27–3.13 (m, 1H), 2.86–2.78 (m, 2H), 2.66–2.60 (m, 2H), 2.45–2.37 (m, 2H), 2.00 (s, 3H), 1.77–1.68 (m, 2H), 1.63–1.51 (m, 6H), 1.32–1.24 (m, 22H); ^{13}C NMR (125 MHz, CD_3OD)(the amide exists as an approximate 1:1 mixture of rotamers) δ 176.6, 176.3, 173.1, 70.0, 68.9, 65.7, 55.0, 53.7, 44.5, 40.0, 39.5, 39.4, 38.1, 37.9, 34.3, 34.0, 32.8, 30.8, 30.73, 30.67, 30.63, 30.5, 30.4, 29.7, 27.0, 26.7, 20.8 ppm.

EXAMPLE VI

This example describes methods for preparing novel pentacyclic intermediates for the preparation of the Crambescidin/Ptilomycalin family of guanidinium alkaloids and congeners. This example further relates to improved chemical synthesis of pentacyclic intermediates for the preparation of the Crambescidin/Ptilomycalin family of guanidinium alkaloids and congeners.

Synthesis

Tethered Biginelli Condensation. The allyl ester was chosen to protect the C(22) carboxylic acid, since this protecting group can be removed in the presence of a guanidinium salt (Overman, L. E.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 2657). Biginelli condensation between compounds **151** (Overman, L. E.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 2657) and **152** (Overman, L. E.; et al. **1995**, *supra*), using the previous conditions (Overman, L. E.; et al. **1995**, *supra*), gave only 30-40% of product compound **153** with poor diastereoselectivity (2:1).

Attention was turned to the integrity of compound **152** (Figure 48). Urea **155** (Figure 48) was synthesized in an improved yield by reaction of precursor amine **154** (Overman, L. E.; et al. **1995**, *supra*) with trimethylsilyl isothiocyanate (Vishnyakova, T. P.; et al. *Russ. Chem. Rev.* **1985**, *54*, 249) (Figure 48). When the ozonolysis of compound **155** was quenched with H_2 and 10% Pd/C, followed by filtration and concentration, a solid product was obtained after 1 h under reduced pressure (0.1 mm) at 23°C. This material, gave superior yields in the Biginelli condensation (60%). Diastereoselectivity, however, was still poor (ds = 2:1). Extensive optimization of reaction conditions showed that in the non-typical solvent trifluoroethanol, the Biginelli condensation proceeded with good diastereoselectivity (ds=6.5:1 (~50%) 0.5 M, ds = 4:1 (80%) 1.7 M). The use of this solvent to improve efficiency and stereoselectivity in Biginelli condensations was recently reported (McDonald and Overman, *J. Org. Chemistry*, **1999**, 64:1520-1528).

Morpholinium acetate was selected as a catalyst for the Biginelli reaction (Renhowe, P. A. Ph.D. Thesis, University of California, Irvine. 1995). An important discovery regarding the use of morpholinium acetate was made during optimization of the Biginelli reaction. After reductive hydrogenation of the ozonolysis product of compound **152**, but prior to filtration and concentration, morpholinium acetate was added to the methanolic solution of compound **152**. The solution was then concentrated to give a viscous oil compound **156** that was characterized by HRMS analysis. Subjection of this oil to Biginelli condensation provided compound **153** in a much improved yield of 80%. Moreover, this modification resulted in

halving the reaction time to 1.5 days.

Synthesis of Enantiopure Iodide Compound 166. Previous synthesis formed iodide **166**, the C(1)–C(7) fragment, in only moderate enantiomeric purity (86% ee) by enantioselective reduction of an ynone precursor (Overman, L. E.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 2657; Renhowe, P. A. Ph.D. Thesis, University of California, Irvine. 1995). A shorter synthesis that provides this intermediate in enantiomeric purity is summarized in Scheme VIII (Figure 34). Diethylzinc addition to aldehydes **159** or **160**, which were synthesized from compounds **157** and **158** respectively, in the presence of a TADDOLate catalyst (Weber, B.; Seebach, D. *Tetrahedron*, **1994**, 50, 7473-7484) gave chiral alcohols **161** and **162** in good yield and >99% ee as determined by GLC analysis of the derived Mosher esters (Seebach, D.; et al. *Helv. Chim. Acta* **1987**, *70*, 954; Seebach, D.; et al. *Chimia* **1991**, 238; Seebach, D.; et al. *Helv. Chim. Acta* **1992**, *75*, 438; Seebach, D.; et al. *Helv. Chim. Acta* **1992**, *75*, 2171; Seebach, D.; et al. *Tetrahedron* **1994**, *50*, 4363; Weber, B.; Seebach, D. *Tetrahedron* **1994**, *50*, 7473). Alcohol **161** was found identical, except for optical rotation, to the intermediate employed in our original synthesis (Overman, L. E.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 2657). Enantiopure **163** was converted to (*S*)-(*Z*)-1-iodo-5-triisopropylsiloxy-3-heptene (Overman, L. E.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 2657). At this point, the TIPS protecting group was deemed unnecessarily robust and was replaced with TBDMS. PMB protection of the primary alcohol allowed for TBDMS protection of the secondary alcohol, thus delivering primary iodide **166** as summarized in Figure 49. Organolithium **167** was generated from **166** by lithium–iodide exchange at –78°C (Dale, J. A.; et al. *J. Org. Chem.* **1969**, *34*, 2543; Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512; Ward, D. E.; Rhee, C. K. *Tettrahedron Lett.* **1991**, *32*, 7165).

Coupling of the C(1)–C(7) Fragment with the Tricyclic Intermediate. This stage of the synthesis was the least satisfactory of the earlier synthesis due to the lability of the aldehyde related to **169** (Overman, L. E.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 2657; Renhowe, P. A. Ph.D. Thesis, University of California, Irvine. 1995). In the present allyl series, when compound **168** was oxidized with the Swern reagent substantial epimerization at C(8)

occurred. No epimerization occurred with Dess-Martin periodinane oxidation (Figure 50). The resulting aldehyde **169** was *O*-methylated according to established protocol (Overman, L. E.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 2657; Renhowe, P. A. Ph.D. Thesis, University of California, Irvine. 1995). However, addition of compound **167** to aldehyde **170**, followed by oxidation of the crude epimeric alcohols, provided ketone **171** in low yield (20-30%). When pure compound **170** or **171** was exposed to either commercial or deactivated silica gel for ~1 h, significant lost of mass (~30%) was observed. This observation partially explains of the low yields for these two steps. An alternative sequence of events was developed to overcome this difficulty (Figure 51).

Addition of 2.2 equivalent of compound **167** to aldehyde **169**, and subsequent oxidation, yielded ketone **172** in an unoptimized yield of 30-40% (46-51% based on consumed compound **169**). Ketone **172** was *O*-methylated, guanylated, deprotected and cyclized to pentacyclic allyl ester **8** (without intermediate purification) in an unoptimized 25-30% overall yield. This sequence should be optimizable and minimizes the loss of material upon silica gel chromatography.

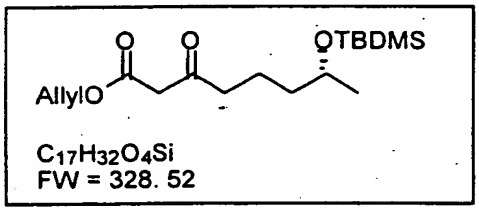
Synthesis of Pentacyclic Acid 7. The allyl ester was successfully removed under standard conditions with Pd(0)/dimedone to furnish pentacyclic acid **7** (Figure 51).

The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

General experimental details: All reactions were carried out under an atmosphere of Ar or N_2 and concentrations were performed under reduced pressure with a BÚchi rotary evaporator. Tetrahydrofuran (THF), Et_2O and CH_2Cl_2 were degassed with Ar then passed through two 4 x 36 inch columns of anhydrous neutral A-2 alumina (8 x 14 mesh; LaRoche Chemicals; activated under a flow of Ar at 350°C for 3h) to remove water. Toluene was degassed with Ar then passed through one 4 x 36 inch column of Q-5 reactant (Englehard; activated under a flow of 5% H_2/N_2 at 250°C for 3 h) to remove O_2 then through one 4 x 36 inch column of anhydrous neutral A-2 alumina (8 x 14 mesh; LaRoche Chemicals; activated under a flow of Ar at 350°C for 3h) to remove water. Triethylamine (Et_3N), pyridine, diisopropylethylamine (*i*-Pr_2NEt), diisopropylamine, and acetonitrile were distilled from CaH_2 at atmospheric pressure. Indicated molarities of organolithium reagents were

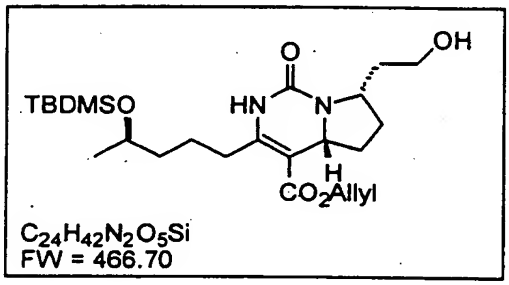
established by titration with menthol/fluorene (Posner, G. H.; Lentz, C. M. *J. Am. Chem. Soc.* **1979**, *101*, 934). Instrumentation and Chromatography: 300 MHz ^{1}H and 75 MHz ^{13}C spectra were obtained on a BrÚker QE 300 FT NMR; 500 MHz ^{1}H and 125 MHz ^{13}C NMR spectra were obtained on a BrÚker GN 500 FT NMR or BrÚker Ω 500 FT NMR. ^{1}H NMR chemical shifts are reported as δ values in ppm. Coupling constants are reported in Hz and refer to apparent multiplicities.

Multiplicity is indicated as follows: s (singlet); d (doublet); t (triplet); m (multiplet); app t (apparent t); dd (doublet of doublets) etc. Mass spectra were measured on a MicroMass Analytical 7070E (CI-isobutane) or a MicroMass AutoSpec E (FAB) spectrometer. Infrared spectra were recorded using a Perkin Elmer 1600 FTIR spectrometer. Microanalyses were performed by Atlantic Microlabs, Atlanta, GA. Optical rotations were measured using a JASCO DIP-360 digital polarimeter. TLC and column chromatography were performed using E. Merck silica gel (43-60 μm) with a loading of approximately 30:1 SiO_2:substrate.



(R)-Allyl-7-(t-butyldimethylsiloxy)-3-oxooctanoate (Compound 151). Freshly distilled allyl acetoacetate (5.0 mL, 37 mmol) was added dropwise to a 0°C mixture of hexane-washed NaH (1.73 g, 43 mmol) and dry THF (50 mL). After 10 min, *n*-butyllithium (14.9 mL of a 2.7 *M* solution in hexanes) was then added dropwise and the resulting red solution was maintained for an additional 10 min at 0°C. A solution of compound **150** (4.53 g, 14.4 mmol) and dry THF (20 mL) was then added dropwise. After 20 min at 0°C the reaction mixture was quenched with saturated aqueous NH_4Cl (20 mL). The layers were separated, and the H_2O layer was extracted with Et_2O (2 x 15mL), and the combined organic layers were washed with brine (15 mL), dried ($MgSO_4$) and concentrated. Purification of the residue on silica gel

(20:1 hexanes-EtOAc) provided 2.84 g (60%) of compound **151** as a colorless oil (9:1 mixture of keto and enol forms by ^{1}H NMR analysis): ^{1}H NMR (500 MHz $CDCl_3$) δ 5.86–5.90 (m 1H) 5.31 (d J = 17.0 Hz 1H) 5.23 (d J = 10.5 Hz 1H) 4.60 (d J = 5.7 Hz 2H) 3.76 (dd J = 11.9 6.0 Hz 1H) 3.44 (s 2H) 2.52 (t J = 7.3 Hz 2H) 1.63–1.66 (m 1H) 1.55–1.58 (m 1H) 1.35–1.40 (m 2H) 1.09 (d J = 6.0 Hz 3H) 0.85 (s 9H) 0.02 (s 6H); ^{13}C NMR (125 MHz $CDCl_3$) 202.5 166.9 131.5 118.8 68.2 65.9 49.1 43.1 38.8 25.9 23.7 19.7 18.1 –4.4 –4.7 ppm; IR (film) 2956 2857 1748 1716 1255 1149 836 775 cm^{-1}; $[\alpha]^{25}_{D}$: –12.8° $[\alpha]^{25}_{577}$: –12.9° $[\alpha]^{25}_{546}$: –14.1° $[\alpha]^{25}_{435}$: –23.8° $[\alpha]^{25}_{405}$: –28.1° (c = 1.05 $CHCl_3$). Anal. Calcd for $C_{17}H_{32}O_4Si$: C 62.15: H 9.82. Found: C 62.42; H 9.93.

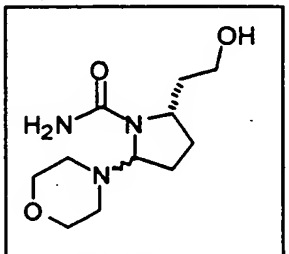


(4aR,7S)-4-(Allyloxycarbonyl)-1,2,4a,5,6,7-hexahydro-7-(2-hydroxyethyl)-3-[(4S)-4-(t-butyldimethylsiloxypentyl)]-1-oxopyrrolo[1,2-c]pyrimidine (153a) and (4aS,7S)-4-(Allyloxycarbonyl)-1,2,4a,5,6,7-hexahydro-7-(2-hydroxyethyl)-3-[(4S)-4-(t-butyldimethylsiloxypentyl)]-1-oxopyrrolo[1,2-c]pyrimidine (Compound 153b). A solution of crude (*S*)-**156** (2.3 g 9 mmol) **151** (2.2 g 6.7 mmol) and trifluoroethanol (4 mL) was heated at 60°C for 2 d. The reaction mixture was quenched by pouring into Et_2O (50 mL) washing with saturated aqueous NH_4Cl (2 x 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) concentrated and purified on silica gel (1:1 hexanes-EtOAc) to yield 2.01 g (64%) of the desired *cis*-Biginelli product **153a** and 0.51 g (16%) of the *trans*-Biginelli product **153b.**

Compound 153a: ^{1}H NMR (500 MHz $CDCl_3$) δ 8.26 (s, 1H) 5.89–5.97 (m 1H) 5.30 (dd J

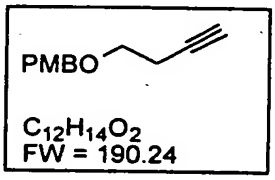
= 16.7 1.2 Hz 1H) 3.22 (dd J = 10.4 1.0 Hz 1H) 4.60 (ddd J = 22.6 13.1 5.9 Hz 2H) 4.26 (dd J = 11.3 4.9 Hz 1H) 4.10–4.14 (m 1H) 3.74–3.80 (m 1H) 3.40–3.67 (m 2H) 2.58 (t J = 7.5 Hz 2H) 2.47–2.52 (m 1H) 2.02–2.11 (m 1H) 1.84 (m 1H) 1.60–1.77 (m 4H) 1.36–1.53 (m 3H) 1.10 (d J = 6.0 Hz 3H) 0.85 (s 9H) 0.02 (s 3H) 0.01 (s 3H); ^{13}C NMR (125 MHz $CDCl_3$) δ 165.2 155.0 152.9 132.3 118.5 102.2 68.6 64.8 59.0 58.3 52.2 39.4 38.9 31.1 30.6 29.8 25.8 25.0 23.7 18.1 –4.4 –4.7 ppm; IR (film) 3450 3225 3095 2954 1682 1626 1431 1111 835 776 cm^{-1}; $[\alpha]^{25}_{D}$: –28.6° $[\alpha]^{25}_{577}$: –30.0° $[\alpha]^{25}_{546}$: –36.8° $[\alpha]^{25}_{435}$: –97.7° $[\alpha]^{25}_{405}$: –138° (c = 2.20 $CHCl_3$). Anal. Calcd for $C_{24}H_{42}N_2O_5Si$: C 61.77; H 9.07; N 6.00. Found: C 61.66; H 9.15; N 5.92.

Compound 153b: ^{1}H NMR (500 MHz $CDCl_3$) δ 8.52 (s 1H) 5.87–5.94 (m 1H) 5.28 (d J = 17.5 Hz 1H) 5.21 (d J = 10.4 Hz 1H) 4.60 (ddd J = 13.4 7.5 6.0 Hz 2H) 4.39–4.45 (m 1H) 4.34 (dd J = 10.5 4.6 Hz 1H) 3.77 (dd J = 11.5 5.7 Hz 1H) 3.56–3.66 (m 2H) 2.66–2.71 (m 1H) 2.49–2.54 (m 1H) 2.42–2.46 (m 1H) 2.08 (dd J = 20.7 8.7 Hz 1H) 1.76–1.81 (m 1H) 1.62–1.67 (m 1H) 1.37–1.54 (m 6H) 1.10 (d J = 6.04 Hz 3H) 0.8 (s 9H) 0.02 (s 3H) 0.01 (s 3H); ^{13}C NMR (125 MHz $CDCl_3$) 165.3 153.2 150.1 132.4 118.2 98.7 68.5 64.7 58.9 57.3 53.7 38.9 38.3 35.1 31.3 28.2 25.9 24.5 23.6 18.1 –4.5 –4.7 ppm; IR (film) 3442 3256 3100 2930 2897 1708 1668 1634 1463 1236 1082 736 cm^{-1}; $[\alpha]^{25}_{D}$: –26.3° $[\alpha]^{25}_{577}$: –26.8 $[\alpha]^{25}_{546}$: –29.3° $[\alpha]^{25}_{435}$: –54.7° $[\alpha]^{25}_{405}$: –122° (c = 2.30 $CHCl_3$). Anal. Calcd for $C_{24}H_{42}N_2O_5Si$: C 61.77; H 9.07; N 6.00. Found: C 61.75; H 9.10; N 5.96.



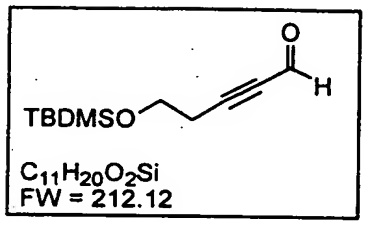
$C_{11}H_{21}N_3O_3$
FW = 243.31

Compound (156). Ozone was bubbled through a solution of compound **155** (1.74 g 9 mmol) and MeOH (50 mL) at –78°C until the solution was saturated (blue color appeared and persisted for ~10 min). Nitrogen was then bubbled through the solution to dissipate the excess ozone. 10% ,d/C (0.6 g) was added to the colorless solution and the reaction mixture was maintained at –78°C under 1 atm of H_2. After 30 min the cooling bath was removed morpholinium acetate (2.0 g 13 mmol) was added and the reaction mixture was allowed to warm to 23°C. After 4 h the reaction mixture was dried ($MgSO_4$) filtered and the filtrate was concentrated. The resulting residue was diluted with trifluoroethanol (30 mL) and concentrated to give a yellow oil which was used without further purification: MS (CI) *m/e* cald for $C_{11}H_{21}N_3O_3$ 243.1583 found 243.1588 (M).

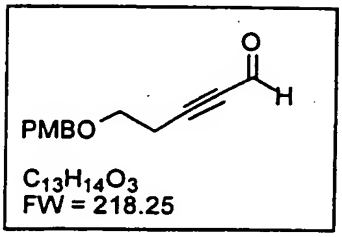


1-(4-Methyoxybenzyloxy)-3-butyne (Compound 158). According to established procedures (Takaku H. et al.; *Tetrahedron Lett.* **1983** 24 5363; Nakajima N.; et al. *Tetrahedron Lett.* **1988** 29 4139) TfOH (1.6 mL 18 mmol) was added dropwise to a 0°C solution of ,MBOC(=NH)CCl_3 (169.3 g 0.6 mol) 3-butyn-1-ol (67 g 0.66 mol) dry Et_2O (600 mL). After 30 min the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ (100 mL) the layers were separated the aqueous layer was extracted with Et_2O (50 mL) and the combined organic layers are washed with brine (50 mL) dried ($MgSO_4$) and concentrated. The resulting residue was diluted with hexanes (300 mL) filtered through a plug of silica gel concentrated and stirred under vacuum (0.1 mm Hg) at 50°C for 12 h yielding **158** (~100%) which is used without further purification. ^1H NMR (500 MHz $CDCl_3$) δ 7.28 (d J = 8.4 Hz 2H) 6.89 (d J = 8.4 Hz 2H) 4.49 (s 2H) 3.80 (s 3H) 3.58 (t J = 7.0 Hz 2H) 2.49 (dt J = 7.0 2.7 Hz 2H) 2.00 (t J = 2.6 Hz 1H); ^{13}C NMR (125 MHz

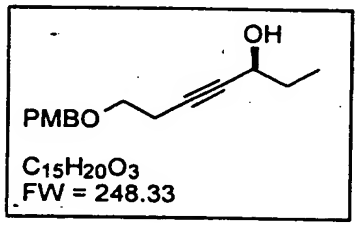
$CDCl_3$) δ 159.2 130.0 129.3 113.7 81.3 72.5 69.2 67.8 55.2 19.8 ppm; IR (film) 3292 3001 2936 2863 1614 1514 823 cm^{-1}; Anal. Calcd for $C_{12}H_{14}O_2$: C 75.76; H 7.42. Found: C 75.60; H 7.49.



5-(t-Butyldimethylsiloxy)-2-pentynal (Compound 159). A hexane solution of *n*-BuLi (2.5 M 4.8 mL) was added to a –78°C solution of compound **157** (2.0 g 10.9 mmol) in dry THF (20 mL). After 10 min the reaction mixture was placed into an ice bath and dry DMF (5 mL) in THF (20 mL) was added. After 30 min at 0°C the reaction mixture was quenched by pouring into a vigorously stirred solution of 5% H_2SO_4 (20 mL). After 1 h the layers were separated the H_2O layer was extracted with Et_2O (3 x 15 mL) and the combined organic layers were washed with saturated aqueous $NaHCO_3$ (1 x 15 mL) and brine (1 x 15 mL) dried ($MgSO_4$) and concentrated. ,urification of the residue on silica gel (4:1 hexanes-EtOAc) provided 0.921 g (55%) of compound **159** as a slightly yellow oil: ^1H NMR (500 MHz $CDCl_3$) δ 9.17 (s 1H) 3.79 (t *J* = 6.7 Hz 2H) 2.62 (t *J* = 6.7 Hz 2H) 0.9 (s 9H) 0.1 (s 6H); ^{13}C NMR (125 MHz $CDCl_3$) δ 177.0 96.2 82.3 60.6 25.8 23.5 18.3 -5.3 -5.4 ppm; IR (film) 2930 2205 1671 1111 cm^{-1}; HRMS (CI isobutane) *m/e* calcd for $C_{11}H_{20}O_2Si$ 212.1232 found 197.0998 (M – CH_3).



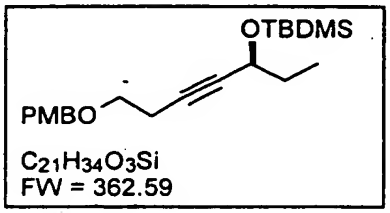
5-(4-Methoxybenzyloxy)-2-pentynal (compound 160). A hexane solution of *n*-BuLi (2.5 M 9.34 mL mL) was added to a –78°C solution of compound **158** (4.04 g 21.2 mmol) in dry THF (100 mL). After 10 min the reaction mixture was placed into an ice bath and dry DMF (10 mL) in THF (100 mL) was added. After 30 min at 0°C the reaction mixture was quenched by pouring into a vigorously stirred solution of 5% aqueous H_2SO_4 (100 mL). After 1 h the layers were separated the H_2O layer was extracted with Et_2O (3 x 30 mL) and the combined organic layers were washed with saturated aqueous $NaHCO_3$ (1 x 30 mL) and brine (1 x 30mL) dried ($MgSO_4$) and concentrated. ,urification of the residue on silica gel (4:1 hexanes-EtOAc) provided 2.55 g (55%) of compound **160** as a slightly yellow oil: ^{1}H NMR (500 MHz $CDCl_3$) δ 9.16 (s 1H) 7.26 (d J = 8.5 Hz 2H) 6.88 (d J = 8.6 Hz 2H) 4.48 (s 2H) 3.79 (s 3H) 3.61 (t J = 6.7 Hz 2H) 2.69 (t J = 6.7 Hz 2H); ^{13}C NMR (125 MHz $CDCl_3$) δ 177.0 159.2 129.6 129.3 113.8 95.7 81.9 72.7 66.5 55.2 20.6 ppm; IR (film) 3002 2865 2205 1668 1514 824 cm^{-1}; Anal. Calcd for $C_{13}H_{14}O_3$: C 71.54; H 6.47. Found: C 71.42; H 6.54.



(5S)-Hydroxy-1-(4-methoxybenzyloxy)-3-heptyne (compound 162). According to the general procedure of Seebach (Ti(O*i*-,r)4 (0.50 mL 1.68 mmol) was added to a 23°C solution of (4*R* 5*R*)-2 2-Dimethyl–α,α,αэ,αэ–тetra(naphth-2-yl)-1 3-dioxolan-4 5-dimethanol (1.12 g 1.67 mmol) and dry toluene (15 mL). After 3 h solvent was removed under reduced pressure (0.1 mm). The resulting residue was dissolved in dry Et_2O (33 mL) and the reaction vessel was cooled to –26°C whereupon Ti(O*i*-,r)$_4$ (3.0 mL 10 mmol) compound **160** (1.83 g 8.37 mmol) and Et_2Zn (9.1 mL of a 1.1 *M* solution in toluene)were

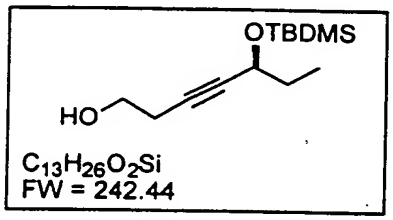
added. After 18 h at –26°C the reaction mixture was quenched with saturated aqueous NH_4Cl (1mL) dried ($MgSO_4$) filtered through Celite®, concentrated and the resulting residue was purified on silica gel (4:1 hexanes-EtOAc) to provide 1.833 g (88%)□of compound **162** as a colorless oil: ^{1}H NMR (500 MHz $CDCl_3$) δ 7.25 (d J = 8.4 Hz 2H) 6.86 (d J = 8.4 Hz 2H) 4.46 (s 2H) 4.26 (t J = 6.4 Hz 1H) 3.78 (s 3H) 3.53 (t J = 7.0 Hz 2H) 2.58 (s 1H) 2.49 (dt J = 7.0 1.5 Hz 2H) 1.66 (m 2H) 0.97 (t J = 7.4 Hz 3H); ^{13}C NMR (125 MHz CDCl3) δ 159.1 129.9 129.2 113.7 82.3 81.7 72.4 67.9 63.5 55.1 30.9 19.9 9.4 ppm; IR (film) 3418 2965 1613 1514 1249 823 733 cm^{-1}; $[\alpha]^{25}_{D}$: –3.2° $[\alpha]^{25}_{577}$: –3.6° $[\alpha]^{25}_{545}$: –4.0° $[\alpha]^{25}_{435}$: –6.5° $[\alpha]^{25}_{405}$: –7.7° (c = 2.35 $CHCl_3$). Anal. Calcd for $C_{15}H_{20}O_3$: C 72.55; H 8.12. Found: C 72.26; H 8.14.

Following the general procedure of Ward (Ward D. E.; Rhee C. K. *Tetrahedron Lett.* **1991** *32* 7165) compound **162** (23 mg) was treated with (*R*)–α–methoxy–α–(triflouromethyl)phenylacetic acid chloride [(*R*)-MT,ACl] to give the corresponding (*R*)-MT,A ester. Capillary GC analysis [150°C to 200°C/2.0°C min^{-1} t_R **162**-(*R*)-MT,A = 21.13 min t_R *ent* -**162**-(*R*)-MT,A = 20.69 min] showed a ratio for 99.7:0.3 of **162**-(*R*)-MT,A and *ent* -**162**-(*R*)-MT,A.



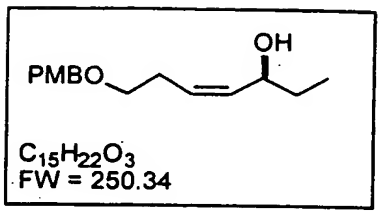
(5S)-(t-Butyldimethylsiloxyl)-1-(4-methoxybenzyloxy)-3-heptyne. TBSCl (1.08 g 7.2 mmol) was added in portions over 15 min to a 23°C solution of imidazole (0.53 g 7.8 mmol) compound **162** (1.48 g 6 mmol) and dry DMF (5 mL). After standing at 23°C for 2 h the solution was poured into 20 mL H_2O and extracted with Et_2O (4 x 20 mL). The combined organic layers were washed with brine (20 mL) dried ($MgSO_4$) and concentrated. The crude

oil was placed under vacuum (0.1 mm) overnight to provide 2.16 g (100%) of the desired product as a colorless oil which was used without further purification: ^{1}H NMR (500 MHz $CDCl_3$) δ 7.28 (d J = 8.5 Hz 2H) 6.89 (d J = 8.5 Hz 2H) 4.48 (s 2H) 4.28 (dt J = 6.2 1.7 Hz 1H) 3.80 (d J = 1.6 Hz 3H) 3.56 (dt J = 7.2 1.52 Hz 2H) 2.51 (dt J = 7.2 1.7 Hz 2H) 1.66 (appt J = 7.0 Hz 2H) 0.96 (dt J = 7.3 1.3 Hz 3H) 0.91 (d J = 1.4 Hz 9H) 0.13 (d J = 1.4 Hz 3H) 0.11 (d J = 1.4 Hz 3H); ^{13}C NMR (125 MHz $CDCl_3$) δ 159.2 130.2 129.2 113.73 82.8 80.9 72.6 68.3 64.4 55.2 31.9 25.8 20.1 18.3 9.2 -4.5 -5.0 ppm; IR (film) 2930 1614 1514 1249 1099 837 cm^{-1}; $[\alpha]^{25}_{D}$: −34.5° $[\alpha]^{25}_{577}$: −35.0° $[\alpha]^{25}_{546}$: −40.9° $[\alpha]^{25}_{435}$: −69.5° $[\alpha]^{25}_{405}$: −83.5° (c = 5.35 $CHCl_3$). Anal. Calcd for $C_{21}H_{34}O_3Si$: C 69.56; H 9.45. Found: C 69.49; H 9.50.

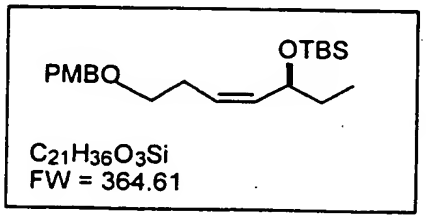


(S)-(5)-(t-Butyldimethylsiloxy)-3-heptynol. A solution of (5*S*)-(*t*-butyldimethylsiloxyl)-1-(4-methoxybenzyloxy)-3-heptyne (0.17 g 0.46 mmol) DDQ (0.16 g 0.68 mmol) and 20:1 CH_2Cl_2-H_2O (3 mL) was maintained at 23°C for 2 h. The reaction mixture was quenched by pouring into Et_2O (25 mL) and washing with saturated aqueous $NaHCO_3$ (2 x 5 mL) followed by brine (5 mL). The organic layer was dried ($MgSO_4$) concentrated and the resulting residue was purified on silica gel (4:1 hexanes-EtOAc) to provide 88 mg (80%)□of the desired product as a colorless oil: ^{1}H NMR (500 MHz $CDCl_3$) δ 4.26 (t J = 6.3 Hz 1H) 3.69 (t J = 6.6 Hz 2H) 2.47 (dt J = 6.3 1.6 Hz 2H) 1.98 (s 1H) 1.61–1.67 (m 2H) 0.94 (t J = 7.4 Hz 3H) 0.9 (s 9H) 0.11 (s 3H) 0.10 (s 3H); ^{13}C NMR (75 MHz $CDCl_3$) δ 84.0 80.6 64.4 61.1 31.9 25.8 23.1 18.3 9.7 –4.6 –5.0 ppm; IR (film) 3388 2958 2858 1472 1256 1059 cm^{-1}; HRMS (EI-GCMS) *m/e* calcd for $C_{12}H_{26}O_2Si$ 242.1701 found 242.1655

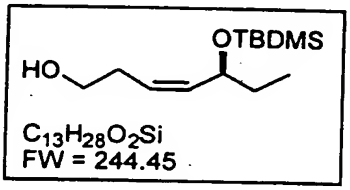
(M); $[\alpha]^{25}_{D}$:–46.0° $[\alpha]^{25}_{577}$: –48.1° $[\alpha]^{25}_{546}$: –54.5° $[\alpha]^{25}_{435}$: –93.2° $[\alpha]^{25}_{405}$: –111.5° (*c* = 1.4 $CHCl_3$).



(3Z,5S)-1-(4-Methyloxybenzyloxy)-3-hepten-5-ol (compound 163). A mixture of compound **162** (13.8 g 55.6 mmol) freshly distilled quinoline (138 mL 1.20 mmol) Lindlar's catalyst (,d/$CaCO_3$ poisoned with ,bO 1.29 g) and dry 3:1 hexanes -EtOAc (138 mL) was maintained at 23°C under 1 atm H_2 for 3 d. This mixture was then filtered through a plug of Celite the plug was washed with 3:1 hexanes-EtOAc (400 mL) and the elutent was concentrated to yield 13.9 g (100%) of compound **163** which was used without further purification: ^1H NMR (300 MHz $CDCl_3$) δ 7.24 (d *J* = 8.4 2H) 6.86 (d *J* = 8.5 2H) 5.51-5.55 (m 2H) 4.44 (s 2H) 4.30 (appq *J* = 7.0 1H) 3.79 (s 3H) 3.34-3.53 (m 2H) 2.25- 2.55 (m 2H) 2.21-2.24 (m 1H) 1.41-1.64 (m 2H) 0.86 (t *J* = 7.4 3H) ; ^{13}C NMR (75 MHz $CDCl_3$) δ 159.1 135.2 129.8 129.3 128.6 113.7 72.7 68.61 68.0 55.2 29.7 28.3 9.7 ppm; IR (film) 3421 3007 2961 2860 1613 1514 1249 1094 821 cm^{-1}; $[\alpha]^{25}_{D}$ = -17.9° $[\alpha]^{25}_{577}$ = -17.8° $[\alpha]^{25}_{546}$ = -20.6° $[\alpha]^{25}_{435}$ = -35.0° $[\alpha]^{25}_{405}$ = -42.6° (*c* = 2.2 $CDCl_3$). Anal. Calcd for $C_{15}H_{22}O_3$: C 71.97; H 8.86. Found: C 71.97; H 8.90.

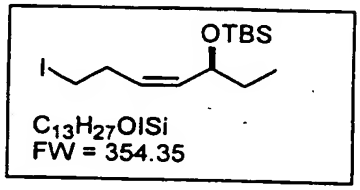


(3Z,5S)-4-(t-Butyldimethylsiloxy)-1-(4-methoxybenzyloxy)-3-heptene (compound 164). TBSCl (0.51 g 3.4 mmol) was added in portions over 15 min to a solution of imidazole (0.48 g 7.0 mmol) compound **163** (0.7 g 2.8 mmol) and dry DMF (1.4 mL) at 23°C. After standing at 23°C for 2 h the solution was poured into 20 mL H_2O and extracted with Et_2O (4 x 20 mL). The combined organic layers were washed with brine (20 mL) dried ($MgSO_4$) and concentrated. The crude oil was placed under vacuum (0.1 mm Hg) overnight to provide 1.02 g (100%) of **164** as a colorless oil: ^{1}H NMR (300 MHz $CDCl_3$) δ 7.26 (d J = 8.3 Hz 2H) 6.88 (d J = 8.5 Hz 2H) 5.34-5.46 (m 2H) 4.45 (s 2H) 4.38 (appq 1H) 3.80 (s 3H) 3.45 (t J = 7.0 Hz 2H) 2.35 (m 2H) 1.38-1.56 (m 2H) 0.84-0.88 (m 12H) 0.05 (s 3H) 0.02 (s 3H) ; ^{13}C NMR (75 MHz $CDCl_3$) 159.1 135.8 130.4 129.2 124.6 113.7 72.6 70.2 69.4 55.2 31.3 28.6 25.8 18.2 9.8 -4.4 -4.8 ppm; IR (film) 2967 2856 1616 1514 1464 1250 1098 836 cm^{-1}; $[\alpha]^{25}_{D}$ = 14.4° $[\alpha]^{25}_{577}$ = 15.7 ° $[\alpha]^{25}_{546}$ = 18.4° $[\alpha]^{25}_{435}$ = 33.5° $[\alpha]^{25}_{405}$ = 42.4° (c = 1.98 $CHCl_3$). Anal. Calcd for $C_{21}H_{36}O_3Si$: C 69.18; H 9.95. Found: C 69.30; H 10.03.

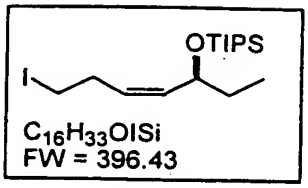


(S)-(Z)-5-(t-Butyldimethylsiloxy)-3-heptenol (compound 165). A solution of compound **164** (0.17 g 0.47 mmol) DDQ (0.16 g 0.68 mmol) and 20:1 CH_2Cl_2-H_2O (3 mL) was maintained at 23°C for 2 h. The reaction mixture was quenched by pouring into Et_2O (25 mL) and washing with saturated aqueous $NaHCO_3$ (2 x 5 mL) followed by brine (5 mL). The organic layer was dried ($MgSO_4$) concentrated and the resulting residue was purified on silica gel (4:1 hexanes-EtOAc) to provide 92 mg (80%) of the desired product as a colorless oil: ^{1}H NMR (500 MHz $CDCl_3$) δ 5.46–5.50 (m 1H) 5.30–5.36 (m 1H) 4.31 (dd J = 14.6 6.7 Hz 1H) 3.63 (dt J = 6.6 2.1 Hz 2H) 2.29–2.34 (m 2H) 1.94 (s 1H) 1.50–1.60 (m 1H) 1.37–1.24 (m 1H) 0.86 (app s 12H) 0.03 (s 3H) 0.01 (s 3H); ^{13}C NMR (125 MHz

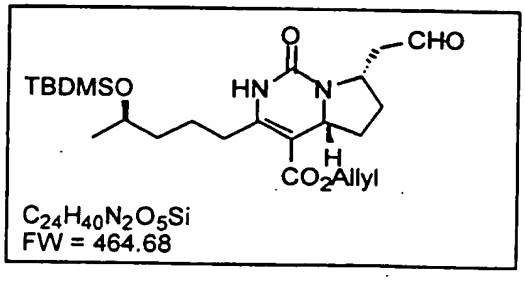
C_6D_6) δ 136.6 125.2 70.4 62.1 31.8 31.7 26.1 18.4 10.0 –4.0 –4.1 ppm; IR (film) 3354 3014 2958 1460 1253 1050 cm^{-1}; $[\alpha]^{25}_{D}$: 20.8° $[\alpha]^{25}_{577}$: 21.3° $[\alpha]^{25}_{546}$: 25.2° $[\alpha]^{25}_{435}$: 47.4° $[\alpha]^{25}_{405}$: 59.7° (*c* = 2.30 $CDCl_3$). Anal. Calcd for $C_{13}H_{28}O_2Si$: C 63.88; H 11.55. Found: C 63.82; H 11.53.



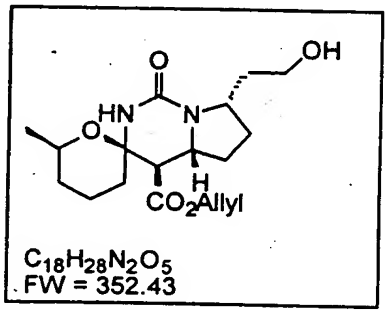
(S)-(Z)-1-Iodo-5-(tert-butyldimethylsiloxy)-3-heptene (compound 166). Following the general procedure of Corey (Singh S. N.; et al. *J. Am. Chem. Soc.* **1987** *109* 6187; Garegg ,. J.; Samuelsson B. *J. Chem. Soc., Perkin Trans. 1* **1980** 2866) iodine (2.09 g 8.24 mmol) was added in portions over 15 min to a 0°C solution of compound **165** (1.83 g 7.49 mmol) ,,h3 (2.03 g 9.0 mmol) imidaz ole (0.61 g 8.99 mmol) and Et_2O-MeCN (3:1 40 mL) and then allowed to warm to 23°C. After 1.5 h the solution was diluted with 1:1 hexanes-EtOAc (200 mL) then filtered through basic alumina (activity-IV) and concentrated. The resulting mixture was flushed though a plug of silica gel (9:1 hexane-Et_2O) to yield 2.5 g (94%) of the desired product as a colorless oil which was used without any further purification: ^{1}H NMR (300 MHz $CDCl_3$) δ 5.46-5.52 (m 1H) 5.23-5.32 (m 1H) 4.25 (dd *J* = 14.4 6.6 Hz 1H) 3.11-3.16 (m 2H) 2.60-2.68 (m 2H) 1.37-1.60 (m 2H) 0.84-0.89 (m 12H); ^{13}C NMR (75 MHz $CDCl_3$) 136.2 127.0 70.2 32.0 31.3 25.8 18.2 9.8 4.6 -4.3 -4.7 ppm; IR (film) 3612 2957 2530 2857 1699 1650 1252 cm^{-1}; $[\alpha]^{25}_{D}$ = 21.9° $[\alpha]^{25}_{577}$ = 22.6° $[\alpha]^{25}_{546}$ = 26.2° $[\alpha]^{25}_{435}$ = 49.5° $[\alpha]^{25}_{405}$ = 62.2° (*c* = 2.00 $CHCl_3$). Anal. Calcd. for $C_{13}H_{27}OISi$: C 44.07; H 7.68. Found: C 44.24; H 7.64.



(S)-(Z)-1-Iodo-5-(triisopropylsiloxy)-3-heptene. Following the general procedure of Corey (Singh S. N.; et al. *J. Am. Chem. Soc.* **1987** *109* 6187; Garegg ,. J.; Samuelsson B. *J. Chem. Soc., Perkin Trans. 1* **1980** 2866) iodine (0.80 g 3.5 mmol) was added in portions over 15 min to a 0°C solution of (*S*)-(*Z*)-5-(triisopropylsiloxy)-3-heptenol (0.900 g 3.14 mmol) ,,h 3 (0.78 g 3.5 mmol) imidazole (0.24 g 3.5 mmol) and Et_2O-MeCN (3:1 5 mL) and then allowed to warm to 23°C. After 1.5 h the solution was diluted with 1:1 hexanes-EtOAc (50 mL) then filtered through basic alumina (activity-IV) and concentrated. The resulting mixture was flushed though a plug of silica gel (9:1 hexane-Et_2O) to yield 1.29 g (97%) of the desired product as a colorless oil which was used without any further purification: ^{1}H NMR (500 MHz $CDCl_3$) δ 5.49-5.53 (m 1H) 5.28-5.32 (m 1H) 4.41 (dd *J* = 7.1 5.9 Hz 1H) 3.10-3.14 (m 2H) 2.59-2.66 (m 2H) 1.58-1.62 (m 1H) 1.48–1.52 (m 1H) 1.05 (s 21H) 0.86 (t *J* = 7.4 Hz 3H); ^{13}C NMR (125 MHz CDCl3) 136.2 126.9 70.0 32.2 31.6 18.1 12.3 9.3 4.4 ppm; IR (film) 3012 2942 1464 1105 883 cm^{-1}; $[\alpha]^{25}_{D}$: 22.8° $[\alpha]^{25}_{577}$: 24.4° $[\alpha]^{25}_{546}$: 23.7° $[\alpha]^{25}_{435}$: 53.1° $[\alpha]^{25}_{405}$: 65.8° (*c* = 1.2 $CHCl_3$). Anal. Calcd for $C_{16}H_{33}OSiI$: C 48.48; H 8.39. Found: C 48.63; H 8.49.



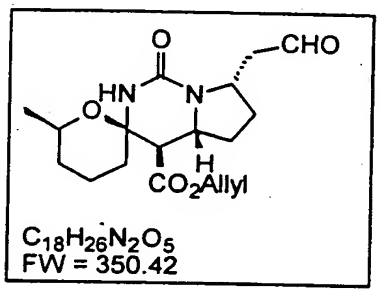
(4aR,7S)-4-(Allyloxycarbonyl)-1,2,4a,5,6,7-hexahydro-3-[(4S)-4-(t-butyldimethylsiloxypentyl)]-1-oxo-7-(2-oxyethyl)pyrrolo[1,2-c]pyrimidine. Dess-Martin periodinane (Dess D. B.; Martin J. C. *J. Org. Chem.*, **1983** 48 4155) (0.50 g 1.2 mmol) was added to a 23°C solution of compound **153a** (0.46 g 1 mmol) and CH_2Cl_2 (10 mL). After 1 h the reaction mixture was poured into Et_2O (50 mL) and washed with saturated aqueous $Na_2S_2O_3$ (2 x10 mL) 1 N NaOH (2 x 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) concentrated and purified on silica gel (1:1 hexanes-EtOAc) to yield 0.404 g (81%) of desired product as a colorless oil: ^{1}H NMR (500 MHz $CDCl_3$) δ 9.73 (s 1H) 8.21 (s 1H) 5.88–5.95 (m 1H) 5.30 (dd J = 16.7 1.2 Hz 1H) 5.22 (d J = 10.4 Hz 1H) 4.60 (ddd J = 22.6 13.1 5.9 Hz 2H) 4.29–4.35 (m 2H) 3.75–3.78 (m 1H) 3.15 (dd J = 16.7 3.8 Hz 1H) 2.57–2.62 (m 1H) 2.52–2.56 (m 2H) 2.44–2.51 (m 1H) 2.11–2.14 (m 1H) 1.62–1.73 (m 2H) 1.57–1.61 (m 1H) 1.53–1.56 (m 1H) 1.39–1.45 (m 2H) 1.09 (d J = 6.0 Hz 3H) 0.85 (s 9H) 0.02 (s 3H) 0.01 (s 3H); ^{13}C NMR (75 MHz $CDCl_3$) δ 200.0 165.3 152.6 152.1 132.3 118.5 101.0 68.4 64.8 58.2 51.0 48.4 39.1 31.1 30.6 29.6 25.8 24.6 23.7 18.1 –4.5 –4.7 ppm; IR (film) 3218 3096 2955 2856 2730. 1722. 1679 1630 1439 1253 836 775 734 cm^{-1}; $[\alpha]^{25}_{D}$: –35.4° $[\alpha]^{25}_{577}$: –35.5° $[\alpha]^{25}_{546}$: –44.6° $[\alpha]^{25}_{435}$: –61.6° $[\alpha]^{25}_{405}$: 19.8° (c = 1.85 $CHCl_3$). Anal. Calcd for $C_{24}H_{40}N_2O_5Si$: C 62.04; H 8.68; N 6.03. Found: C 61.75; H 8.68; N 6.00.



$C_{18}H_{28}N_2O_5$
FW = 352.43

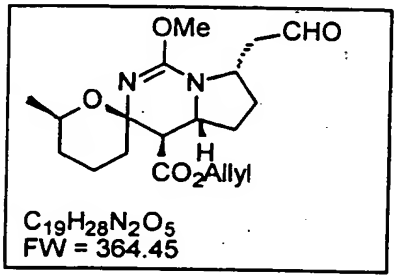
(3R,4R,4aR,6'R,7S)-4-(Allyloxycarbonyl)-1,2,4a,5,6,7-hexahydro-7-(2-hydroxyethyl)-1-oxopyrrolo[1,2-c]pyrimidine-3-spiro-6'-(2'-methyl)-3',4',5',6'-tetrahydro-2H-pyran

(compound 168). A solution of compound **153a** (0.486 g, 1.04 mmol), PPTS (0.262 g, 1.04 mmol), and MeOH (20mL) was heated at 50°C for 5 h. The resulting solution was concentrated, flushed through a plug of silica gel (20:1 EtOAc-MeOH), and concentrated. The resulting residue was dissolved in a solution of $CHCl_3$ and *p*-TsOH (45 mg, 0.24 mmol), which was maintained at 23°C for 1 h, then poured into Et_2O (60 mL). The solution was washed with saturated aqueous $NaHCO_3$ (2 x 10 mL), brine (10 mL), dried ($MgSO_4$), and concentrated to yield 0.345 g **168** (94%) as a slightly yellow oil, which was used without further purification: ^1H NMR (500 MHz, $CDCl_3$) δ 6.26 (s, 1H), 5.84–5.92 (m, 1H), 5.32 (d, J = 17.4 Hz, 1H), 5.22 (d, J = 10.4 Hz, 1H), 4.62 (ddd, J = 21.1, 12.5, 6.2 Hz, 2H), 4.33 (s, 1H), 4.13–4.33 (m, 1H), 4.02 (dt, J = 11.1, 5.0 Hz, 1H), 3.77–3.80 (m, 1H) 3.53–3.58 (m, 2H), 2.32 (d, J = 11.1 Hz, 1H), 2.13–2.23 (m, 2H), 1.98–2.03 (m, 1H), 1.52–1.74 (m, 8H), 1.05–1.09 (m, 1H), 1.02 (d, J = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4, 154.6, 131.7, 118.5, 82.5, 66.2, 65.4, 59.2, 55.3, 54.4, 53.6, 39.7, 32.4, 32.2, 30.3, 29.4, 21.7, 18.6 ppm; IR (film) 3297, 3084, 2934, 1731, 1659, 1633, 1480, 1012, 733 cm^{-1}; $[\alpha]^{25}_D$: 139°, $[\alpha]^{25}_{577}$: 145°, $[\alpha]^{25}_{546}$: 166°, $[\alpha]^{25}_{435}$: 285°, $[\alpha]^{25}_{405}$: 345°, (c = 2.25, $CHCl_3$). Anal. Calcd for $C_{18}H_{28}N_2O_5$: C, 61.34; H, 8.00; N, 7.95. Found: C, 61.08; H, 8.08; N, 7.78.



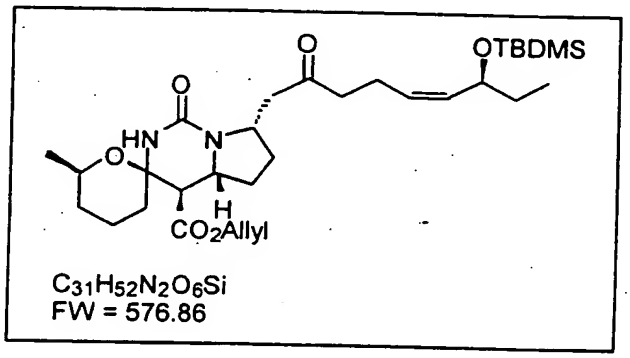
(3R,4R,4aR,6'R,7S)-4-(Allyloxycarbonyl)-1,2,4a,5,6,7-hexahydro-1-oxo-7-(2-oxyethyl)-pyrrolo[1,2-c]pyrimidine-3-spiro-6'-(2'-methyl)-3',4',5',6'-tetrahydro-2H-pyran (compound 169). Dess-Martin periodinane (Dess, D. b.; Martin, J. C. *J. Org. Chem.* **1983**, 48, 4155) (0.72 g, 1.7 mmol) was added to a 23°C solution of compound **168** (0.500 g, 1.42 mmol) and CH_2Cl_2 (35 mL). After 1 h the reaction mixture was poured into Et_2O (100 mL) and washed

with saturated aqueous $Na_2S_2O_3$ (2 x10 mL), 1 N NaOH (2 x 20 mL), brine (20 mL). The organic layer was dried ($MgSO_4$), concentrated, and purified on silica gel (EtOAc; 20:1 EtOAc-MeOH) to yield 0.404 g (81%) of compound **169** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.66 (s, 1H), 6.84 (s, 1H), 5.79–5.87 (m, 1H), 5.28 (d, J = 17.1 Hz, 1H), 5.16 (d, J = 10.5 Hz, 1H), 4.59-4.63 (m, 1H) 4.51-4.55 (m, 1H) 4.32 (dd, J = 12.5, 7.9 Hz, 1H), 4.00 (dt, J = 11.2, 4.7 Hz, 1H), 3.76 (dd, J = 11.1, 5.9 Hz, 1H), 3.09 (dd, J = 16.7, 4.1 Hz, 1H), 2.33 (dd, J = 16.7, 7.9 Hz, 1H), 2.28 (d, J = 11.2 Hz, 1H), 2.09-2.13 (m, 1H), 1.96–2.07 (m, 2H), 1.82 (dd, J = 25.8, 12.2 Hz, 1H), 1.39–1.64 (m, 5H), 1.00–1.04 (m, 1H), 0.97 (d, J = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 200.2, 168.3, 153.3, 131.6, 118.2, 82.1, 66.0, 65.2, 54.7, 53.8, 51.8, 48.6, 32.1, 31.9, 29.5, 29.3, 21.5, 18.2 ppm; IR (film) 3229, 3079, 2932, 2730, 1732, 1660, 1651, 1470, 1014, 733 cm^{-1}; $[\alpha]^{25}_{D}$: 110°, $[\alpha]^{25}_{577}$: 115°, $[\alpha]^{25}_{546}$: 132°, $[\alpha]^{25}_{435}$: 238°, $[\alpha]^{25}_{405}$: 299°, (c = 2.50, $CHCl_3$). Anal. Cacld for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.80; H, 7.53; N, 8.06.



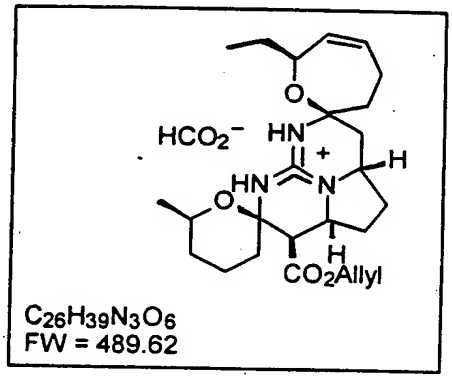
(3R,4R,4aR,6'R,7S)-4-(Allyloxycarbonyl)-3,4,4a,5,6,7-hexahydro-1-methoxy-7-(2-oxyethyl)-pyrrolo[1,2-c]pyrimidine-3-spiro-6'-(2'-methyl)-3',4',5',6'-tetrahydro-2H-pyran (compound 170). A solution of compound **169** (0.285 g, 0.813 mmol), MeOTf (0.368 mL, 3.26 mmol), 2,6-di-*t*-butyl-4-methylpyridine (0.25 g, 1.22 mmol), and dry CH_2Cl_2 (5 mL) was maintained at 23°C for 5 h. The solution was then poured into Et_2O (40 mL) and washed with 1 N NaOH (2 x 10 mL) and brine (10 mL), dried (Na_2SO_4), filtered, concentrated, and the resulting residue was purified on 10% pH 7 phosphate buffered silica gel (4:1 hexanes-EtOAc; 3:1 hexanes-EtOAc) to yield 200 mg (68%) of compound **170** as a colorless oil: ^{1}H

NMR (500 MHz, $CDCl_3$) δ 9.68 (s, 1H) 5.87–5.95 (m, 1H) 5.35 (d, J = 17.4 Hz, 1H) 5.21 (d, J = 10.5, 1H) 4.64-4.68 (m, 1H) 4.58-4.61 (m, 1H) 4.29–4.33 (m, 1H) 4.03–4.07 (m, 1H) 3.87 (dt, J = 11.1, 5.3 Hz, 1H) 3.66 (s, 3H) 2.76–2.80 (m, 1H) 2.34–2.39 (m, 1H) 2.14–2.24 (m, 2H) 2.02–2.10 (m, 2H) 1.96 (dt, J = 12.8, 3.9 Hz, 1H), 1.66 (dt, J = 12.8, 6.5 Hz, 1H) 1.51–1.55 (m, 2H) 1.39–1.46 (m, 1H) 1.34 (d, J = 12.6 Hz, 1H) 1.05 (ddd, J = 13.4, 11.6, 4.0 Hz, 1H) 0.97 (d, J = 6.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 200.5, 170.6, 150.3, 132.2, 117.8, 84.9, 65.6, 64.9, 56.6, 54.3, 52.5, 51.8, 50.0, 35.0, 33.6, 29.9, 29.2, 22.2, 19.4 ppm; IR (film) 2932, 2725, 1727, 1636, 1455, 1393, 1017, 754 cm^{-1}; $[\alpha]^{25}_{D}$: 177°, $[\alpha]^{25}_{577}$: 185°, $[\alpha]^{25}_{546}$: 213°, $[\alpha]^{25}_{435}$: 387°, (c = 2.00, $CHCl_3$). Anal. Calcd for $C_{19}H_{28}O_5N_2$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.36; H, 7.77; N, 7.52.



(3R,4R,4aR,6'R,7S)-4-(Allyloxycarbonyl)-1,2,4a,5,6,7-hexahydro-1-oxo-7-[(7S)-(Z)-2-oxo-7-(t-butyldimethylsiloxy)-5-nonenyl]-pyrrolo[1,2-c]pyrimidine-3-spiro-6'-(2'-methyl)-3',4',5',6'-tetrahydro-2H-pyran (compound 172). *t*-BuLi (1.83 mL, 1.44 M in hexanes) was added to a –78°C solution of compound **166** (439 mg, 1.24 mmol), Et_2O (5 mL), and hexanes (7.5 mL). After 20 min, the solution is cannulated into a –78°C solution of compound **169** (0.20 g, 0.57 mmol) and THF (10 mL). After 5 min, the reaction mixture is quenched with saturated aqueous NH_4Cl (10 ml). The layers were separated, and the aqueous layer extracted with Et_2O (10 mL). The combined organic layers were washed with brine (5 mL), dried ($MgSO_4$), and concentrated to yield a slightly yellow oil, which was used without further purification.

The crude oil, Dess-Martin periodinane (48 mg, 0.11 mmol), and CH_2Cl_2 (10 mL) were maintained at 23°C for 45 min. The reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ (10 mL), saturated aqueous $NaHCO_3$ (10 mL) and Et_2O (30 mL). The layers were separated and the organic layer was washed with brine (5 mL), dried ($MgSO_4$), and concentrated to yield a slightly yellow oil, which was purified on silica gel (1:1 hexanes-EtOAc) to obtain 99 mg (30%) of the desired product as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.31 (s, 1H), 5.84-5.90 (m, 1H), 5.29-5.34 (m, 2H), 5.17-5.22 (m, 2H), 4.64-4.68 (m, 1H), 4.56-4.59 (m, 1H), 4.25-4.30 (m, 2H), 4.02 (dt, J = 11.2, 5.0 Hz, 1H), 3.77-3.81 (m, 1H), 3.38 (dd, J = 16.6, 2.1 Hz, 1H), 2.33-2.45 (m, 2H), 2.20-2.27 (m, 4H), 2.00-2.26 (m, 3H), 1.22-1.77 (m, 8H), 1.06-1.09 (m, 1H), 1.02 (d, J = 6.1 Hz, 3H), 0.79-0.83 (m, 12H), 0.00 (s, 3H), -0.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 208.2, 168.5, 153.1, 135.0, 131.6, 126.7, 118.4, 52.1, 69.8, 66.0, 65.3, 54.9, 53.8, 53.0, 46.4, 42.6, 32.2, 32.1, 29.4, 28.9, 25.7, 21.7, 21.6, 18.4, 18.1, 9.7, -4.5, -4.9 ppm; IR (film) 3226, 3079, 2931, 1736, 1717, 1652, 1472, 1375, 1255, 1084, 1015 cm^{-1}; $[\alpha]^{25}_{D}$ = 64.6°, $[\alpha]^{25}_{577}$ = 67.9°, $[\alpha]^{25}_{546}$ = 78.1°, $[\alpha]^{25}_{435}$ = 144°, $[\alpha]^{25}_{405}$ = 177°, (c = 2.25, $CHCl_3$). Anal. Calcd. for $C_{31}H_{52}O_6N_2Si$: C, 64.55; H, 9.06; N, 4.87. Found: C, 64.39; H, 8.98; N, 4.77.

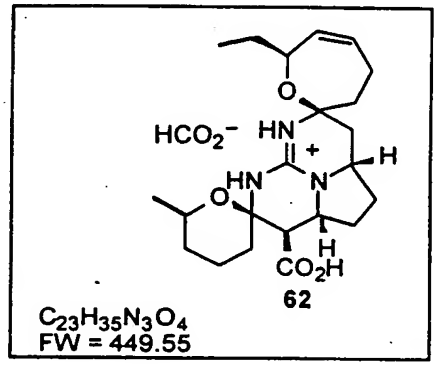


Allyl ester Compound 8. A solution of compound **172** (110 mg, 0.19 mmol), MeOTf (0.37 μL, 3.3 mmol), 2,6-di-*t*-butyl-4-methylpyridine (10 mg, 0.05 mmol), and dry CH_2Cl_2 (8 mL)

was maintained at 23°C for 12 h. The solution was then poured into Et_2O (30 mL) and washed with 1 N NaOH (2 x 5 mL) and brine (5 mL) dried (Na_2SO_4) filtered concentrated and the resulting residue was used without further purification.

Anhydrous NH_3 was bubbled through a 0°C solution of the crude residue and MeOH (25 mL) in a resealable tube. After 15 min the tube was sealed and heated to 50°C. After 2 d the solution was concentrated and the crude residue was used without further purification.

The crude residue TsOH (95 mg 0.50 mmol) and $CHCl_3$ (10 mL) was maintained at 23°C. After 8 h the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (2 mL). The layers were separated and the aqueous was extracted with Et_2O (2 x 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to yield a slightly yellow oil which was purified on silica gel (10:1:0.1 $CHCl_3$:*i*-,rOH:HCO $_2$H) to obtain 23 mg (25%) of the desired product as a slightly yellow oil.

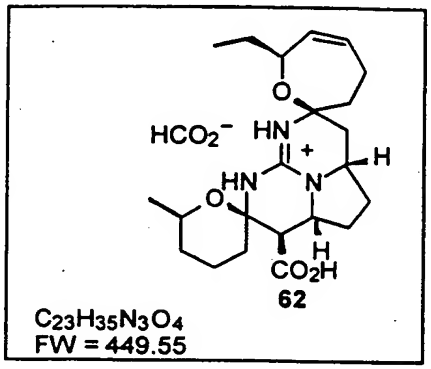


62

$C_{23}H_{35}N_3O_4$
FW = 449.55

Pentacyclic Acid Compound 7. A solution of compound **8** (23 mg 0.05 mmol) ,d(„h_3) (4 mg 3 µmol) dimedone (35 mg 0.25 mmol) and THF (1 mL) was maintained at 23°C. After 10 min the reaction mixture was concentrated and purified on silica gel (10:1:0.1 $CHCl_3$:*i*-,rOH:HCO_2H – 4:1 $CHCl_3$:HCO_2H) to obtain 3 mg (13%) of the desired product as a slightly yellow oil: HRMS (FAB) *m/z* 404.2549 calcd for $C_{22}H_{34}O_4N_3$ found 404.2541.

organic layers were dried ($MgSO_4$), and concentrated to yield a slightly yellow oil, which was purified on silica gel (10:1:0.1 $CHCl_3$:*i*-PrOH:HCO_2H) to obtain 23 mg (25%) of the desired product as a slightly yellow oil.



62

$C_{23}H_{35}N_3O_4$
FW = 449.55

Pentacyclic Acid Compound 7. A solution of compound **8** (23 mg, 0.05 mmol), $Pd(PPh_3)$ (4 mg, 3 μmol), dimedone (35 mg, 0.25 mmol), and THF (1 mL) was maintained at 23°C. After 10 min, the reaction mixture was concentrated and purified on silica gel (10:1:0.1 $CHCl_3$:*i*-PrOH:HCO_2H – 4:1 $CHCl_3$:HCO_2H) to obtain 3 mg (13%) of the desired product as a slightly yellow oil: HRMS (FAB) *m/z* 404.2549 calcd for $C_{22}H_{34}O_4N_3$, found 404.2541.

EXAMPLE VII

An Improved Synthesis of Pentacyclic Acid

This Example provides an improved method for synthesizing pentacyclic acid compounds. Chemical synthesis procedures are as described above for Example VI. A convergent synthetic strategy for the guanidinium alkaloids is shown in Figure 46.

Figure 52 depicts the synthesis strategy for a new method of preparing pentacyclic acid compounds and the compounds produced, e.g. compounds **176 and 177,** using compound **173** as starting material. Compound 61 is an urea compound obtained as shown in Figures 53, 54

and 55 as follows: 3-butynol (compound **178**) is converted to the *p*-methoxybenzyl (PMB) ether **179** (Figure 53). The alkyne of compound **179** was deprotonated with n-buthyl lithium at –40°C and the resulting acetylide treated with anhydrous DMF to provide ynal **180** in 90% yield, after quenching the intermediate –aminoalkoxide into aqueous phosphate buffer (Journet et al., Tetrahedron Lett., 1988, 39:6427). The C3 stereocenter was introduced by the method of Weber and Seebach (Singh et al., *J. Am. Chem. Soc.*, 1987, 109:6187) through condensation of ynal **180** with Et_2Zn in the presence of (-)-TADDOL (20 mol%) and Ti(Oi-Pr)$_4$ to give (S)-**181** in 94% yield and >98% ee. This asymmetric transformation was reliably performed on a 45 g scale. Propargylic alcohol **181** was protected as the triisopropylsilyl (TIPS) ether and the alkyne partially hydrogenated with Lindlar's catalyst to provide *cis* alkene **182**. The PMB-protecting group was oxidatively removed with DDZ and the resulting alcohol was converted to iodide **183** (Kitamura et al., *Org. Synth.*, 1992, 71:1) in an overall yield of 89% from **181**.

Enantiopure methyl R-3-hydroxy-7-methyloct-6-enoate (Kitamura et al., *Org. Synth.*, 1992, 71:1) was converted to amide **185** in 88% yield by reaction with N,O-dimethylhydroxylaminde hydrochloride according to the procedure of Weinreb (Garigipati et al., *J. Am. Chem. Soc.*, 1985, 107:7790) followed by protection of the secondary alcohol as the triethylsilyl (TES) ether (Figure 54). Iodide **183** was converted to the corresponding lithium reagent and coupled with **185** to generate dienone **186** in 60-70% yield. Masking the C8 carbonyl of **186** as the ketal was necessary to prevent a ß-hydroxy elimination, which occurred under the Mitsunobu conditions employed to install the ß-amino functionality. Ketalization was sluggish, however, when the ß-hydroxy group was protected, so optimized reaction conditions were found which cleaved the TES group, did not promote the ß-hydroxy elimination of the intermediate ß- hydroxy ketone and promoted ketalization. Optimized ketalization conditions involved treatment of **186** with orthoester **187** (Roush and Gillis, *J. Org. Chem.*, **1980**, 45:4283-4287; Baganz and Domascke, *Chem. Ber.*, **1958**, 91:650-653) and 1,3-propanediol in the presence of Amberlyst-15 to provide ketal **188** in 80% yield. Mitsunobu displacement of the secondary alcohol with azide followed by reduction to the amine provided compound **189** in 77% yield from compound **188**.

Condensation of amine **189** with TMSNCO yielded urea **190** in 89% yield (Figure 55). Amine **189** is used to prepare pentacyclic compound **177** as shown in Figure 52.

These results demonstrate a method that permits preparation of both pentacyclic compounds 7 and **177**. Having access to both types of compounds, allows side chains to be attached either before or after epimerization of the ester of the pentacyclic compound.

EXAMPLE VIII

This example describes the in vitro screening of 60 tumor cell lines against the compounds of the invention: ptilomycalin A, isocrambescidin 800 trihydrochloride, triacetylcrambescidin 800 chloride, crambescidin 657 hydrochloride, crambescidin 800 trihydrochloride, triacetylisocrambescidin 800 chloride, and 13-epiptilomycalin A to determine anti-tumor activity.

The screening methods utilized the National Cancer Institute (NCI) DTP Human Tumor Cell Line Screen protocol as described by Monks et al., J. Nat'l. Cancer Inst. 83:757-766 (1991); and Boyd In "*Cancer Drug Discovery and Development, Vol. 2; Drug Development; Preclinical Screening, Clinical Trial and Approval,* Humana Press, 1997, pp 23-43. The origins and processing of the cell lines used are described in Alley et al., Cancer Res., 1988, 48:589-601; Shoemaker et al., Prog. Clin. Biol. Res., 1988, 276:265-286; and Stinson et al., Proc. Am. Assoc. Cancer Res., *1989, 30:613.*

In brief, in the screen protocol, cell suspensions were diluted depending on cell type and the expected target cell density (approximately 5000-40,000 cells per well) into a 96 well microtiter plate. Inoculates were preincubated for 24h at 37 □C for stabilization. Dilutions at twice the intended test concentrations were added at time zero in 100 μL aliquots to the microtiter plate wells. Test compounds were evaluated at five 10-fold dilutions. Routine test concentrations have the highest well concentration at 10E-4M, but for the standard agents, the highest well concentration used depended on the agent used. Incubations lasted 48 hours in

5% CO_2 atmosphere and 100% humidity. The cells were assayed by Sulforhodomine B assay as described by Rubenstein et al., JNCI, 1990, 82:1113-1118 and Skehan et al., JNCI, 1990, 82: 1107-1112. Optical densities were read with a plate reader and the data processed using a microcomputer into special concentration parameters.

NCI renamed the IC50 value, the concentration that causes 50% growth inhibition the "GI50" value to emphasize the correction for the cell count at time zero; thus GI50 is the concentration of test drug where 100 X (T-T0)/(C-TO) – 50 (Boyd et al., In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*, Vleriote et al., Eds., Kluwer Academic, Hingham, MA, 1992, pp 11-34; and Monks et al., JNCI, 1991, 83, 757-766. The optical density of the test well after a 48 hr period of exposure to the test compound is "T", the optical density at time zero is TO and the control optical density is "C." The "50" is called the GI50PRCNT, a T/C-like parameter that can have values from +100 to –100. The GI50 also measures the growth inhibitory power of the test compound. The TGI is the concentration of test drug where 100 X (T-T0)/(C-T0) =0. Thus, the TGI signifies a cytostatic effect. The LC50 which signifies a cytotoxic effect, is the concentration of the test compound where 100 X (T-T0)/T0=-50. The control optical density is not used in the calculation of LC50.

These concentration parameters are interpolated values. The concentrations giving G150PRCNT values above and below the reference values (e.g. 50 for G150) are used to make interpolations on the concentration axis. Currently, about 45% of the G150 records in the database are "approximated." In 42% of the records, the G150PRCNT for a given cell line does not go to 50 or below. For mean graph purposes, the value assumed for the G150 in such a case is the highest concentration tested (HICONC). Similar approximations are made when the G150 cannot be calculated because the G150PRCNT does not go as high as 50 or above (3% of total). In this case, the lowest concentration tested is used for the G150. Corresponding approximations are made for the TGI and for the LC50.

The results of the tumor cell screening are shown in the mean graphs of 56-62.

The mean graphs are a presentation of the in vitro tumor cell screen results developed by the NCI to emphasize differential effects of test compounds on various human tumor cell lines (Boyd et al., In *Cancer: Principles and Practice of Oncology*, DeVita et al., Eds., Lippincott, Philadelphia, PA, 1989, Vol. 3, pp. 1-12; Paull et al., JNCI, 1989, 81:1088-1092; and Paull et al., Proc. Am. Assoc. Cancer Res., 1988, 29:488. The mean graph bar graphs depict patterns created by plotting positive and negative values generated from a set of G150, TGI or LC50 values. The positive and negative values are plotted against a vertical line that represents the mean response of all the cell lines in the panel to the test compound. Positive values project to the right of the vertical line and represent cellular sensitivities to the test agent that exceed the mean. Negative values project to the left and represent cell sensitivities to the test compound that are less than the average value. The positive and negative values, called "deltas", are generated from the G150 data (or TGI or LC40 data) by a three-step calculation. The G150 value for each cell line tested against a test compound is converted to its log10 G150 value. The $\log_{10}$ G150 values are averaged. Each $\log_{10}$ G150 value is subtracted from the average to create the delta. Thus, a bar projecting 3 units to the right denotes that the G150 (orTGI or LC50) for that cell line occurs at a concentration 1000 times less than the average concentration required for all the cell lines used in the experiment. Thus the cell line is usually sensitive to that compound. If for a particular compound and cell line it was not possible to determine the desired response parameter by interpolation, the bar length shown in either the highest concentration tested (and the listed $\log_{10}$ of the response parameter will be preceded by a ">") or the lowest concentration tested (and the listed $\log_{10}$ will be preceded by a "<"). The values at either limit (> or <) are also calculated in the mean used for the meangraph. Therefore, the mean used in the meangraph may not be the actual mean of the G150 for instance. For this reason, this value is referred to as the MgMID (meangraph midpoint).

These results demonstrate that certain cancer cell lines are sensitive to ptilomycalin A, triacetylcrambescidin 800 chloride, crambescidin 657 hydrochloride, crambescidin 800 trihydrochloride, and 13-epiptilomycalin A. Isocrambescidin 800 trihydrochloride and triacetylisocrambescidin 800 chloride display a lesser effect on the cell lines tested.